

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/22077> holds various files of this Leiden University dissertation.

Author: Piana, Chiara

Title: Adherence to antiretroviral combination therapy in children : what a difference half a day makes...

Issue Date: 2013-10-31

ADHERENCE TO ANTIRETROVIRAL COMBINATION THERAPY IN CHILDREN.
WHAT A DIFFERENCE HALF A DAY MAKES...

Chiara Piana

Ph.D. Thesis, Leiden University, October 2013

ADHERENCE TO ANTIRETROVIRAL COMBINATION THERAPY IN CHILDREN

WHAT A DIFFERENCE HALF
A DAY MAKES...

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 31 oktober 2013
klokke 13:45 uur

door

Chiara Piana

geboren te Acqui Terme, Italië
in 1982

Promotor	Prof. Dr. M. Danhof
Co-promotor	Prof. Dr. O.E. Della Pasqua
Referenten	Prof. Dr. S. Khoo (University of Liverpool) Prof. Dr. G. Pons (Université Paris Descartes)
Overige Leden	Prof. Dr. P.H. van der Graaf Prof. Dr. C.A.J. Knibbe Prof. Dr. J. Burggraaf Prof. Dr. T. Hankemeier Prof. Dr. A.P. Ijzerman

“It always seems impossible until it’s done”

Nelson Mandela

ai miei genitori

The research leading to this thesis has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 201057 and was performed at the Division of Pharmacology of the Leiden Academic Center for Drug Research, Leiden University, Leiden, The Netherlands.

The printing of this thesis was financially supported by:

Leiden Academic Center for Drug Research
J.E. Jurriaanse Stichting
GlaxoSmithKline
Janssen
Boehringer Ingelheim B.V.
Merck Sharp & Dohme B.V.
Gilead Sciences Netherlands B.V.

Cover and layout by Marta Klement, Atelier Lukes
Printed by GVO printers & designers B.V., Ede, The Netherlands

ISBN: 978-90-9027783-7

©2013 Chiara Piana (chiarapiana82@libero.it)

No part of this thesis may be reproduced or transmitted in any form or by any means without written permission of the author.

TABLE OF CONTENTS

Section 1	General Introduction	9
Chapter 1	Highly Active Antiretroviral Therapy in children: the disease, the drug and the patient: current issues and potential solutions	11
Chapter 2	Scope and intent of the investigation	45
Section 2	Sample size, covariate distribution and predictive performance of pharmacokinetic models	57
Chapter 3	Covariate effects and population pharmacokinetics of lamivudine in hiv-infected children	59
Chapter 4	Population pharmacokinetics of abacavir in infants, toddlers and children	79
Chapter 5	Influence of covariate distribution on model predictive performance	95
Section 3	Simplified dosing regimens in children	113
Chapter 6	A model-based approach for the evaluation of once daily dosing of lamivudine in hiv-infected children	115
Chapter 7	Suitability of once daily dosing abacavir in children	129
Section 4	Forgiveness to poor adherence	145
Chapter 8	Forgiveness of non-adherence for different classes of antiretroviral drugs in children	147
Chapter 9	Impact of non-adherence to antiretroviral combination therapy in HIV-infected children	165
Chapter 10	Forgiveness of non-adherence to once vs. twice daily lamivudine/abacavir in combination with efavirenz in HIV-infected children	181
Section 5	Conclusions and perspectives	197
Chapter 11	Summary, conclusions and perspectives	199

SECTION I

General introduction

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN CHILDREN

The disease, the drug and the patient: current issues and potential solutions

Chiara Piana, Meindert Danhof and Oscar Della Pasqua

Submitted for publication

SUMMARY

Despite the enormous progresses observed in paediatric Highly Active Antiretroviral Therapy (HAART) in the last decades, a high percentage of children continue to experience treatment failure due to development of drug resistance, inadequate dosing and poor adherence. This review is aimed at exploring the current status of antiretroviral therapy in children with focus on the interaction between disease, drug and patient behaviour, all of which are strongly correlated and determinants of treatment outcome. With respect to the disease, an overview of viral characteristics and of the available antiretroviral drug classes currently combined to avoid development of resistance is provided. Taking into account pharmacokinetic and pharmacodynamic properties, we show the advantages and limitations of existing methodologies for dosing recommendation. Finally, the role of the patient is scrutinised: a detailed definition of adherence to therapy is provided, together with the main strategies used to enhance treatment compliance in children. The importance of adherence is also highlighted in terms of its implication for the development of resistance, which has been shown to differ for each class of antiretroviral drugs.

After having identified some of the challenges which need to be overcome to decrease viral failure in children, we propose the use of a model-based approach for exploring forgiveness of non-adherence, which may allow simplification of current dosing regimens taking into consideration inadequate compliance and its implication for efficacy and drug resistance. In conjunction with clinical trial simulations, we demonstrate that it is possible to evaluate relevant clinical scenarios and predict treatment outcome of simplified dosing regimens of antiretroviral drugs in hypothetical populations of HIV-infected children

1.1. BACKGROUND

Paediatric HIV infection is a world-wide public health challenge disproportionately affecting children in the poorest parts of the world, where access to therapy is still quite limited. Major advances occurred during the past 15 years, such as effective prophylaxis and treatment in HIV-infected women, and administration of highly active antiretroviral therapy (HAART) to those babies who are infected (1). Early antiretroviral treatment has dramatically modified the course of HIV infection in children, reducing mortality by fivefold or more and resulting in high survival rates in adulthood (2;3). However, one of the greatest challenges for children living with HIV is maintaining effective antiretroviral treatment for life. A European study which looked at more than a thousand children on antiretroviral treatment found that 12 percent of children experienced treatment failure of three classes of drugs after 5 years, over two times the rate of adults (4).

Some of the reasons that lead to earlier treatment failure in children include a lack of choice of antiretroviral drugs for children, difficulties with adherence and inadequate dosing, together with the risk of running out of drug options sooner in case of drug resistance, and the need for psychosocial support - particularly during adolescence. From a therapeutic perspective, a comprehensive overview is required of the factors which influence treatment outcome and may lead to clinical failure, primarily involving the disease, the drug and the patient's behaviour towards therapy. An extensive evaluation of such factors will be performed in the next paragraphs. Based on such an evaluation, specific goals and effective strategies in clinical practice can be identified, which may contribute to overcome existing issues and in turn to reduce the number of HIV-infected children experiencing viral failure.

The disease: why is combination therapy needed?

At present, a combination of at least three antiretroviral drugs from at least two drug classes is recommended for initial therapy in adults and in children. The antiretroviral classes currently approved in children are NRTIs (nucleoside reverse transcriptase inhibitors), NNRTIs (non-nucleoside reverse transcriptase inhibitors) and PIs (protease inhibitors).

The mechanism of action of NRTIs and NNRTIs is based on the inhibition of reverse transcriptase, a viral DNA polymerase enzyme that retroviruses need to reproduce, while PIs block the HIV protease, an enzyme used by the virus to cleave nascent proteins for final assembly of new virions (5).

There are several causes related to the mechanism of infection and replicative capacity of the virus which elucidate the need for drug combination in HIV treatment: (i) the virus is able to replicate at a very fast rate ($T_{1/2}$ approximately 1 day) once a cell has been infected; therefore one drug may be able to only decrease the rate of this process but not to interrupt it; (ii) given that viral replication depends on different enzymes which are the targets of the antiretroviral drugs, the possibility to reach two targets at the same time increases the chance of stopping HIV and

protecting new cells from infection; (iii) the virus can infect different types of cells in different parts of the body; each drug differs in how well the virus can be attacked in these different cells; (iv) combination of anti-HIV drugs may overcome or delay the development of drug resistance (6;7). Drug resistance may emerge because of the replication program of HIV, which is rapid and error prone (mutation rate ca. 3×10^{-5} mutations/base/replication cycle), resulting in large and genetically diverse populations in vivo (8). When HIV is allowed to replicate in the presence of antiretroviral drug concentrations, which are insufficient to exert complete suppression, antiretroviral drug-resistance mutations will almost invariably emerge (9;10). Depending on the site of viral mutations and their impact on viral fitness, different anti-HIV classes show higher or lower barriers to resistance. A combination of different antiretroviral classes is therefore needed to assure complete viral suppression and prevent the development of drug resistance.

In the past years direct nucleic acid sequencing has become a common mechanism to obtain resistance information; commercial genotyping services, as well as systems for laboratory use are available; routine testing with independent panels of resistant viruses is useful to maintaining proficiency in detection of mutations (11;12). Appropriate use of resistance testing provides valuable information useful in constructing regimens for treatment-experienced individuals with viremia during therapy.

To date, two therapeutic options are suggested as first-line choice in children: NNRTI- and PI- based regimens (13). Regarding NNRTI based regimens, only efavirenz (EFV) and nevirapine (NVP) are currently approved for paediatric patients. In children older than 3 years and able to swallow capsules, EFV is preferred as first-line therapy because of its once daily administration, absence of interaction with food and lower incidence of adverse events compared to nevirapine. Regarding PI-based regimens, most recent guidelines agree in recommending lopinavir/ritonavir (LPV/r) as a first line therapy in naïve paediatric patients. This drug is available as liquid formulation and appears to be safe and effective in children with regards to virological suppression and the increase in CD4 count.

Table 1 Antiretroviral drugs currently approved in children (BID: twice daily, QD: once daily)

ARV class	Drug	Half-life	IC ₅₀	Dose
NRTI	Zidovudine	Serum 0.9-1.4 hrs Intracellular 3-4 hrs(15)	0.003-0.013 mcg/mL (16)	180-240mg/m ² -BID (17)
NRTI	Lamivudine	Serum 2-6 hrs Intracellular 10-15 hrs(18)	2 nM-15µM(19)	4mg/kg per dose-BID (17)
NRTI	Abacavir	Serum 1.5 hrs Intracellular 12-26 hrs(20)	0.26-4.0µM (21)	8-10 mg/kg per dose-BID(17)
NRTI	Didanosine	0.97-1.6 hrs(22)	0.49 µM(23)	<3 months 50mg/m ² -BID >3 months 120 mg/m ² -BID(17).
NRTI	Stavudine	0.9-1.5 hrs(24)	0.009-4µM(25)	1 mg/kg per dose-BID(17)
NRTI	Emtricitabine	8-10 hrs(26)	0.0013-0.64µM(26)	<3 months 3mg/kg-QD >3 months 6mg/kg-QD(17)
NRTI	Tenofovir	17 hrs(27)	0.04 - 8.5 µM(28)	300 mg-QD(> 12 years)(17)
NNRTI	Efavirenz	40-55 hrs after multiple doses(29)	0.51 ng/mL(30)	15 -18.75mg/kg solid form or 19.5 mg/kg syrup-QD (17)
NNRTI	Nevirapine	25-30 hrs(31)	10ng/mL(31)	150-200mg/m2 per dose BID(17)
Boosted PI	Lopinavir/ritonavir	5-6 hrs(32)	0.04- 0.18µg/mL(32)	< 15 kg 12/3 mg/kg-BID > 15 kg 10/2.5 mg/kg -BID(17)
PI	Nelfinavir	3.5-5 hrs(33)	0.06 µM(34)	<10 kg ~75 mg/kg/dose-BID 10 kg to 19.9 kg ~60 mg/kg/dose-BID(17)
PI	Atazanavir	6-7 hrs(35)	2 - 5 nM(36)	<20 kg 150mg + 100mg ritonavir >20 kg <40 kg 200mg + 100mg ritonavir > 40 kg 300mg + 100mg ritonavir(17)

Two drugs from the NRTI class are needed to form the backbone of HAART, with six NR-TIs (zidovudine, didanosine, lamivudine, stavudine, abacavir and emtricitabine) approved for HIV-infected children younger than 13 years of age. Combinations of lamivudine plus abacavir or zidovudine or didanosine are considered the preferred dual NRTIs backbone regimens for initial therapy in children. The selection of the initial regimen of antiretroviral drugs is generally based on several factors, such as comorbid conditions, potential adverse drug effects, potential drug interactions with other medications, results of genotypic drug resistance testing and convenience (e.g. pill burden, dosing frequency). In table 1 an overview of the antiretroviral drugs currently approved in children and their characteristics is provided, whilst table 2 summarises the antiretroviral combinations preferred as initial treatment in HIV-infected children.

An important point to consider which is still quite debatable is the optimal time to start HAART in children. According to the current WHO guidelines, all infants with confirmed HIV infection should be started on HAART, irrespective of the clinical or immunological stage, while for all children 12 months or older clinical and immunological thresholds should be used to identify those who need to start HAART (14)

Table 2 Antiretroviral regimens recommended for initial therapy for HIV infection in children (17)

Preferred Regimens	
Children >14 days and <3 years	Two NRTIs plus lopinavir/ritonavir
Children ≥3 years	Two NRTIs plus efavirenz Two NRTIs plus lopinavir/ritonavir
Children age ≥6 years	Two NRTIs plus atazanavir plus low-dose ritonavir Two NRTIs plus efavirenz Two NRTIs plus lopinavir/ritonavir
2-NRTI Backbone Options for Use in Combination with Additional Drugs	
Preferred	abacavir plus (lamivudine or emtricitabine) (children ≥3 months) tenofovir plus (lamivudine or emtricitabine) (adolescents ≥12 years) zidovudine plus (lamivudine or emtricitabine)

The drug: what is the right dose in HIV-infected children?

Up to now, empirical scaling from adults to children continues to be the mainstream method for dose selection in paediatrics, with adjustment for body weight as the most common approach (37). Although adjustment of drug pharmacokinetic parameters according to body weight or

body surface area (BSA) can occasionally explain the observed exposure differences between adult and paediatric patients, the direction and extent of these differences across age groups, in general, are not predictable. For example, some drugs are eliminated more rapidly or more slowly in younger paediatric patients, compared with adults or older paediatric patients (38). Bioavailability may also differ between children and adults due to differences in transit time or pH. There are extensive physiological changes with pharmacological impact that occur as a child matures from infancy to adulthood, and this process does not occur with precisely predicted timing or magnitude on an individual scale (39;40). Weight-based methods for determining paediatric doses may not account accurately for all variables related to the different stages of maturation and are unlikely to predict consistently the correct dose for each paediatric age group. All these aspects are in strong opposition to the concept of “one size fits all” dosing for children (41) and to the belief of a linear relationship to scale or correct dose for the effect of body weight. Rational considerations on the optimal dose for each child should be taken into consideration. Together with body weight, other confounders such as drug-drug interactions and demographic covariates, i.e. age, gender, body composition, functionality of liver and kidneys and maturation of enzymatic systems throughout the life span from neonates to adults (42) may affect the pharmacokinetics of a drug and consequently its exposure. When selecting the paediatric dose, these potential confounders must be taken into account in order to achieve an adequate exposure and to avoid the risk of toxicity or poor efficacy. A dosing regimen with more than the necessary doses, besides causing toxicity, might also increase the possibility of poor adherence, which is seriously related with occurrence of resistance. Suboptimal concentrations of antiretroviral drugs might as well be very dangerous because they may exert viral selection pressure and thus promote development of drug resistance.

Limited pharmacokinetic data remains one of the major issues in dosing recommendation for HIV-infected children. Although a similar disease progression in children and adults allows for dosing in children based on efficacy data in adults, performing pharmacokinetic trials to assess optimal dosing in children is critical to avoid inadequate exposure. Clinical studies to determine the optimal dosing in children of different ages are also critical to avoid under exposure or toxicity in children belonging to a certain age or weight group. Despite the indisputable need to perform clinical trials in HIV-infected children, one major limitation of such studies must be highlighted. In patients affected by chronic diseases who are obliged to take their medication their whole life, adherence to therapy during the clinical trial may not be a realistic surrogate of patient adherence in real life, due to the limited duration of the study. Awareness is needed with regard to the implications that this may have on treatment outcome. Moreover participation to clinical trials may enhance adherence to treatment in chronic diseases (43), thus the pharmacokinetic profile of the drug might be altered in real life by different patterns of variable adherence.

The patient: adherence to HIV antiretroviral therapy

There are several reasons why antiretroviral treatment fails, of which poor adherence is a leading one (2;44-46). It has been shown that the role covered by the patient in achieving response to treatment is comparable to the one of the drug. A review of 17 studies on paediatric HIV treatment adherence found adherence in HIV-infected children ranging from 49 percent to 100 percent (47). Three-quarters of the studies showed adherence rates of 75 percent. Most of the studies in lower and middle-income countries revealed adherence rates above 75 percent, whereas adherence in higher-income countries was generally below 75 percent.

It is useful to provide an exhaustive definition of the term ‘adherence’ beyond the simple ‘patient’s tendency to follow medical advice’. Two substituent terms must be defined to have a comprehensive understanding of patient adherence: (i) compliance and (ii) persistence (48). The former is defined as ‘the degree of correspondence between the patient’s actual dosing history and the prescribed dosing regimens’. The latter is defined as ‘the time elapsed between the first dose taken and the time of treatment discontinuation’. The term compliance includes also the degree of correspondence between the patient’s actual dosing time and the prescribed dosing time. We handle this component as “quality” of compliance. The different implications on treatment outcome of variable compliance (the patient sporadically misses some doses or takes the drug at different times) and variable persistence (“drug holiday”) are shown in figure 1. It is important to mention that adherence is a critical issue in every chronic treatment, not only in HIV. Numerous studies have investigated the effect of poor adherence in many therapeutic areas such as, hypertension (49), glaucoma (50;51) and osteoporosis (52).

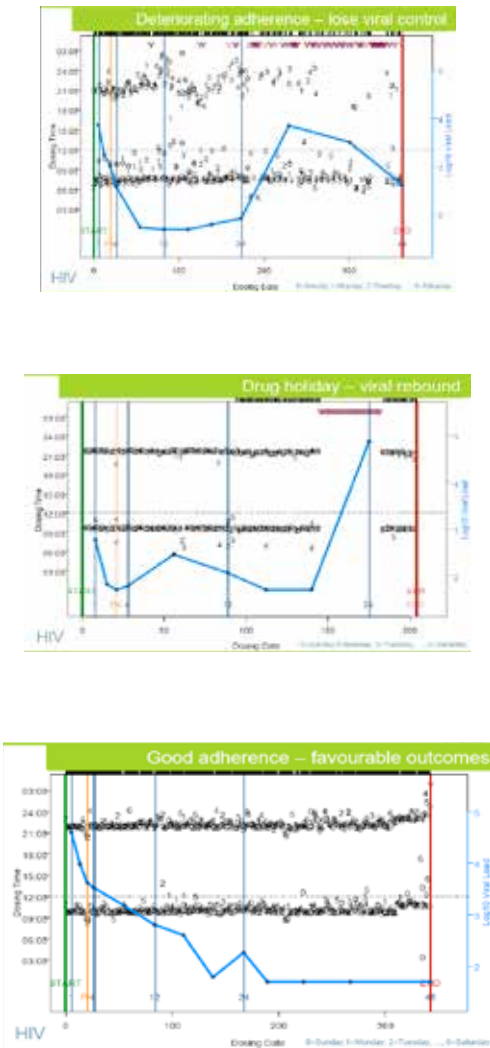


Figure 1 On the upper and central panels two different patterns of non-adherence and consequent effects on viral load are depicted. On the lower panel perfect adherence and consequent decrease in viral load is represented.

Several factors pose specific challenges in children compared to adults (53). First of all young children's adherence depends partly or entirely on a caregiver, who, especially in limited resources countries, may be sick or may need to work when the drug has to be administered (54;55). The identification of someone responsible for the child is difficult, especially when both parents died or are impaired. The expectation that older children should be able to take the medicine independently is often unrealistic (56). Moreover family members often have discrepant perceptions of a child's level of responsibility for medication, especially in families with older children. Another reason which may affect compliance in antiretroviral therapy is the heavy pill burden that sometimes needs to be administered to perinatally infected children in need of savage therapy because of drug resistance and treatment experience (57). These complicated regimens pose greater issues in terms of adherence and therefore may lead to resistance which will create the need for even more complicated regimens. Furthermore, many of the current HIV medicines have an unpleasant taste, especially in syrups and powder form. This can make it difficult for children to take their antiretroviral drugs daily (58).

In the past years several strategies have been adopted to improve adherence in HIV-infected children, mainly based on the education of the caregivers or on peer support, self-monitoring and telephone follow-up (59;60). A brief period of hospitalization may help demonstrating the role of non-adherence on antiretroviral therapy and help identifying possible solutions. Material support such as pillboxes, drug identification charts, daily schedules, diaries and educational materials are provided to explain the schedules, risks and benefits of ART (61). Age-specific developmental-level protocols and teaching materials (e.g., cartoons, stories and drawings) have been developed to educate children about their treatment, their HIV status, and the importance of adherence and medical follow-up.

The possibility to reduce the dosing frequency of antiretroviral drugs is another important strategy to enhance adherence to treatment. It has been demonstrated that decreasing the pill burden and dosing frequency is associated with increased adherence (62-64). Several studies have already been performed to assess the feasibility of reduced dosing frequency of some antiretroviral drugs from three times a day to twice daily or from twice to once daily (65-67). However deep knowledge of the pharmacokinetic and pharmacodynamic properties of a drug is required to understand whether the dosing frequency could be reduced: it has been demonstrated that missing a dose when following once daily dosing regimens may be more dangerous than missing one dose on a twice daily regimen (68).

Adherence-resistance relationship

Failure to take the prescribed dose of antiretroviral drugs leads to ongoing viral replication in the presence of drug and the selection of drug-resistant HIV. It has been shown that poor adherence may increase the risk of drug resistance.

However, the relationship between adherence and development of resistance is not that simple as it may seem and it differs for each class of antiretroviral drugs. Bangsberg *et al.* have used a cohesive model (figure 2) to summarise this complex relationship for each class of antiretroviral drugs currently used as first line therapy (69).

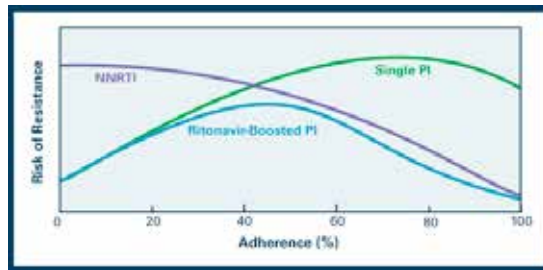


Figure 2 Schematic representation of the model developed by Bangsberg to summarise the relationship between adherence and resistance for each class of antiretroviral drugs. (Modified with permission from Bangsberg *et al.* (69))

According to this model, low levels of adherence are more likely to promote development of resistance to NNRTIs due to their low genetic barriers to drug resistance. On the other hand, higher selection pressure is required for single PIs given the high genetic barriers of this class to resistance; therefore a level of adherence close to perfect is more dangerous than low levels (70). Sporadic missed doses are unlikely to produce high risk combination of actively replicating virus and sub-therapeutic levels in NNRTIs, due to their long half-lives; conversely sub-therapeutic levels may easily be reached after long periods of treatment interruption and therefore the risk of resistance strongly increases. Unboosted PIs have a very short half-life and inferior antiviral efficacy compared to NNRTIs. Their rapid disappearance from circulation during non-adherent periods leads to lower frequency of new mutations compared to NNRTIs, therefore the majority of new PI resistance mutations occur in those with higher adherence rates. Boosted PIs instead have a high degree of antiviral efficacy and longer half-lives than unboosted PIs but shorter than NNRTIs. Their potency discourages the development of resistance in case of missed doses and their intermediate half-lives are impediments to the development of resistance mutations in patients who interrupt the treatment for long periods. The relationship between adherence and resistance is thus more complex than “non-adherence increases the risk of drug resistance”. A good understanding of this relationship is a critical step in drug development and may lead to lower risk of viral failure in HAART.

Assessment of New Highly Active Antiretroviral Therapy

Based on the aforementioned data and considerations, optimal dosing regimen and adherence to prescribed treatment appear to be the main challenges in paediatric antiretroviral therapy and thus constitute the targets for future investigations of researchers and clinicians. The possibility to develop novel drugs with different mechanisms of action able to prevent the development of drug resistance and improve treatment outcome is an alternative solution which is beyond the scope of this study. Undoubtedly dosing frequency reduction may be very advantageous for the patients; however the impact of poor adherence on optimised dosing regimens must be taken into account. The possibility to evaluate which pharmacokinetic and/or pharmacodynamic properties of an antiretroviral drug make it less susceptible to suboptimal adherence and predict treatment outcome might be a powerful tool for dosing regimen optimisation. These considerations rely on an important concept in antiretroviral therapy, which still lacks an established and quantitative measure: forgiveness of non-adherence. Forgiveness of non-adherence is the ability of a drug or regimen to achieve and maintain viral suppression even in case of poor adherence (71). A variety of pharmacological, viral and host properties determine the level of forgiveness of any specific regimen. It is generally used as comparative descriptor of different classes of antiretroviral drugs, based upon the “anchor drug” of the regimen. In 2000 Paterson *et al* showed that extraordinarily high rates of adherence were necessary to achieve viral suppression in a group of HIV-infected patients receiving unboosted indinavir based regimen (45). These findings lead to the “95% rule”, which means that patients must take at least 95% of the prescribed antiretroviral doses in order to control viral replication.

More recent studies have demonstrated that more moderate levels of adherence are needed to achieve and maintain viral suppression in patients treated with NNRTIs and boosted PIs based regimens. These findings gave birth to the evidence that some antiretroviral classes are more forgiving than others. This would be the starting point for future studies and investigations which may provide important information usable in clinical practice. Forgiveness is not defined in a quantitative manner and still lacks a specific scale and detailed thresholds which may influence therapeutic choices and improve the final outcome of the treatment. A quantitative and systematic definition of forgiveness will enable the exploration of new dosing regimens, which will provide advantages for the patients without risks of inadequate efficacy and in turn of drug resistance.

1.2. A MODEL-BASED APPROACH FOR THE EXPLORATION OF NOVEL DOSING REGIMENS

A systematic definition of forgiveness of non-adherence for different drugs and the exploration of alternative dosing regimens requires a quantitative methodology which allows evaluating contemporaneously the effect of disease, drug and patient behaviour.

A model-based approach in which pharmacokinetic-pharmacodynamic models for selected antiretroviral drugs are integrated with a model for viral dynamics may provide a powerful tool to predict efficacy of antiretroviral combination treatments. In addition, the inclusion of a model for patient adherence provides the basis for quantifying the relation between adherence, exposure and drug response as well as the impact of adherence on treatment outcome (72). The evaluation of adherence, however, may not be feasible in clinical practice due to ethical reasons and design issues. Thus an *in silico* approach is required to assess how different patterns of adherence may affect treatment outcome. Quantitative methods can provide insight into how forgiving antiretroviral drugs are and provide the scientific basis for alternative dosing regimens. Similar model-based methods have been already applied in different therapeutic areas, such as statin or antihypertensive therapies (73-75).

The possibility to integrate statistical models that describe disease mechanism, drug behaviour and patient adherence to treatment in clinical trial simulation will be crucial in the exploration of simplified dosing regimens of antiretroviral drugs in *in silico* populations of HIV-infected children based on hypothetical and real-life scenarios (76).

Disease models for viral dynamics

A statistical model that can accurately describe the disease in terms of viral replication and infection is the starting point to predict treatment response of combination antiretroviral therapy and to gain insight into possible mechanisms of treatment resistance (77).

HIV dynamics has been widely studied in the past twenty years and several models of different levels of complexity have been developed (8;78-80). The main advantage of this approach was the possibility to understand, quantify and parameterise viral processes such as replication, infection and death over time (81). For example modelling of viral dynamics has shown that HIV-1 is cleared from chronically infected patients at a rapid rate, with a half-life estimated to 6 hours. Furthermore quantitative estimates of viral parameters suggest that HIV-1 is a rapidly replicating virus and one that could respond to therapy. Finally, modelling has shown that the HIV virus can quickly become resistant to any single drug, particularly to those that require only one mutation to generate resistance. This phenomenon can be anticipated by the fact that every single possible mutation of the viral genome can be expected to occur hundreds or thousands times each day.

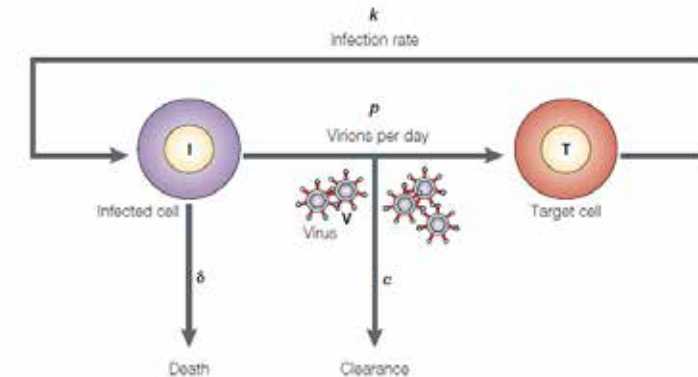


Figure 3 Basic model of viral infection by Perelson. (Modified with permission from Perelson et al. (81))

Figure 3 depicts the basic model commonly used for viral dynamics. This model is widely used also to study the dynamics of hepatitis C virus, hepatitis B virus and cytomegalovirus infections *in vivo*.

The model considers a set of cells susceptible to infection, that is, target cells, T, which, through interactions with virus, V, become infected. Infected cells, I, are each assumed to produce new virus particles at a constant average rate p and to die at rate δ . The average lifespan of a productively infected cell is $1/\delta$ and so if an infected cell produces a total of N virions during its lifetime, the average rate of virus replication per cell, $p = N\delta$. Newly produced virus particles, V, can either infect new cells or be cleared from the body at rate c per virion. This model is defined by a system of three differential equations (equation 1- 3). These equations are applied to obtain minimal estimates for the parameters c and δ . From these estimates it is possible to calculate upper bounds for the half-life of virions in plasma and the half-life of productively infected cells.

$$dT/dt = \lambda - dT - kVT \quad (1)$$

$$dI/dt = kVT - \delta I \quad (2)$$

$$dV/dt = pI - cV \quad (3)$$

It has been observed that viral load decay in plasma takes place with an initial rapid exponential decline of nearly 2 logs of magnitude and continues subsequently with a slower exponential decline that leads to virus falling below the detection limit. The slope of this decline depends on the efficacy of therapy (82-84). The basic model was implemented to interpret this two-phase decay in viral load with the inclusion of more compartments representing a longer-lived popula-

tion of productively infected cells, activation of latently infected cells and release into the blood of virions trapped in tissue reservoirs (85;86).

Equations 1-3 represent the processes of viral infection and replication in absence of an antiretroviral drug. When the effect of an antiretroviral drug or regimen is analysed, the basic model is implemented as defined in equation 4-6, where γ represents the antiviral efficacy (87).

$$dT/dt = \lambda - dT - (1-\gamma) kVT \quad (4)$$

$$dI/dt = (1-\gamma) kVT - \delta I \quad (5)$$

$$dV/dt = \rho I - cV \quad (6)$$

Given that reverse transcriptase inhibitors block the ability of HIV to infect a cell and protease inhibitors cause the production of non-infectious viral particles, the previous equations can be implemented taking into account the different mechanism of action of each class as shown in equations 7-10, where the viral compartment is decomposed in infectious and non-infectious virions (V_I and V_{NI}) and ϵ_{RT} and ϵ_{PI} are the efficacies of RT and PI respectively (8).

$$dT/dt = \lambda - dT - (1 - \epsilon_{RT})kVIT \quad (7)$$

$$dI/dt = (1 - \epsilon_{RT})kVIT - \delta I \quad (8)$$

$$dV_I/dt = (1 - \epsilon_{PI})\rho I - cV_I \quad (9)$$

$$dV_{NI}/dt = \epsilon_{PI} \rho I - cV_{NI} \quad (10)$$

Such models have been widely validated and subsequently used to predict the time course of clinical endpoints and to design novel strategies in HIV treatment (88;89) given that they allow to link drug efficacy to long-term changes in HIV-1 viral load (87;90;91).

Pharmacokinetic-pharmacodynamic modelling in children

Characterisation of the pharmacokinetic-pharmacodynamic relationships is required to assess the correlation between plasma concentrations of antiretroviral drugs with changes in clinical endpoint. In order to define such relationships detailed information on pharmacokinetics and potentially also on pharmacodynamics of antiretroviral drugs in children need to be collected. Given that only a limited number of observations can be obtained in paediatric subjects due to ethical and technical challenges, the population approach using nonlinear mixed effect modelling to obtain pharmacokinetics and pharmacodynamics parameters is the preferred approach (92;93).

The population approach is based on simultaneous analysis of all data of the entire population, while still taking into account that different observations come from different patients. Additionally, the population approach allows not only for the analysis of dense data, but also for sparse (limited number of observations per individual) and unbalanced data (unequal distri-

bution of observations in various parts of the concentration-time profile in the individuals) or a combination of both. Finally, both the inter-individual and intra-individual variability are separately estimated in the dataset using this approach (94). The term “mixed” in nonlinear mixed effects modelling represents a mixture of fixed and random effects. For the fixed effects, a structural model describing the pharmacokinetics or pharmacodynamics is chosen (e.g. a two-compartment model for pharmacokinetics or an Emax model for pharmacodynamics). The random effects quantify the variability that is not explained by the fixed effects and include inter-subject and intra-subject random variability, which are both simultaneously and separately estimated (95). It is often assumed that the variability between subjects follows a normal distribution with a mean of zero and variance ω^2 . Equation 11 is used to describe the relationship between individual and population parameter estimates:

$$\theta_i = \theta_{\text{mean}} * e^{\eta_i} \quad (11)$$

Where θ_i represents the parameter of the i^{th} subject, θ_{mean} the population mean, and η_i the variability between subjects. The residual error is generally described using a proportional error (error is dependent on the concentration, which means a higher absolute error at higher concentrations (Eq.12)) or an additive error (constant for all observations (Eq. 13)) or a combination of both. This means for the j^{th} observed concentration of the i^{th} individual the relation (Y_{ij}):

$$Y_{ij} = c_{\text{pred},ij} * (1 + \epsilon_{ij}) \quad (12)$$

$$Y_{ij} = c_{\text{pred},ij} + \epsilon_{ij} \quad (13)$$

Where c_{pred} is predicted concentration and ϵ_{ij} is a random variable with a mean of zero and a variance of σ^2 .

The structural model uses fixed effects parameters such as clearance and volume of distribution for pharmacokinetics or E_{max} and EC_{50} for pharmacodynamics. The population values for these parameters are called typical values (TV). After selecting the structural model, the statistical submodel, which accounts for the inter-individual as well as the residual variability, is chosen and tested. Information on the inter- and intra-individual or residual variability is of clinical value, because it describes differences in clinical response between and within patients and may therefore provide guidance for rational dose adjustments. In the final step the covariate sub-model is determined, which expresses relationships between covariates and parameters of the structural model (e.g. influence of body weight on volume of distribution or clearance) (96).

Covariate analysis

Differently from adults, developmental changes in children (i.e. metabolising enzyme capacity, renal function, liver flow, body composition) profoundly affect the responses to medications. It

is important that such changes are considered in the context of all other sources of intra- and inter-individual variability resulting from genetic-, environmental- and disease-related factors and drug interactions (97). As shown in figure 4, pharmacokinetic-pharmacodynamic modelling permits the exploration of the influence of different demographic covariates to explain the variability in drug response. As previously mentioned, developmental changes may influence drug exposure and/or drug response. Thus the identification of the demographic covariates which are related to pharmacokinetic or pharmacodynamic parameters is crucial, specifically in children where such covariates may be strongly correlated.

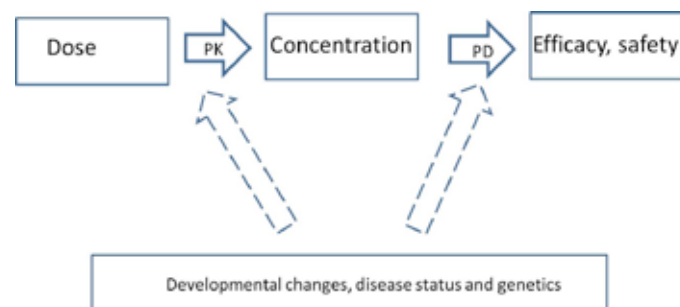


Figure 4 Schematic representation of the relationship between dose and concentration (pharmacokinetics, PK) and between concentration and a pharmacological (side) effect (pharmacodynamics, PD). Important covariates that may affect both the PK and/or PD are body weight, age, disease status (e.g. critically ill versus healthy children) and genetics. (Modified with permission from De Cock et al. (92))

The influence of developmental changes in childhood can be explored primarily by using size and/or age as covariates. Size (body weight) can be incorporated into the model using two different approaches. The first approach, the “allometric size approach”, includes size a priori by using a body weight-based exponential equation with a fixed exponent of 0.75 for clearance and 1 for volume of distribution (98;99). Once size is incorporated in the model using this fixed method, the influence of age is investigated, being the difference between the actual value of the pharmacokinetic parameter and the 0.75 allometric equation.

In the second approach, the “systematic covariate analysis”, body weight is regarded as covariate as any other, which means that the descriptive properties on the pharmacokinetic parameters are evaluated in a systematic covariate analysis as described below (42;100;101). In a systematic analysis, when studying the influence of covariates, scatter plots are used to screen for appropriate covariates to include in the covariate sub-model. Additionally, these plots are used to explore the nature of the influence of the covariate (linear, exponential, allometric, sub-populations, etc.). Likely candidate covariates are then added to the model (forward inclusion).

The influence of each covariate on the parameters is examined separately and compared with the simple model (no covariates). To assess whether the model with the covariate statistically improved the fit to the data, the difference between their objective function values, referred to as the log-likelihood ratio, is calculated. This ratio is assumed to be Chi-squared distributed, which means that a reduction in objective function of 3.84 is considered to be significant ($P < 0.05$) (102).

After all covariates that significantly improved the objective function are added to the simple model, a backward deletion is performed, which means that each covariate is removed from the full model, one at a time. Retaining or removing the covariate is statistically tested by the use of the objective function (Chi-squared test) until each covariate has been tested.

The identification of the correct covariates which are correlated to pharmacokinetic and/or pharmacodynamic parameters in children is particularly challenging due to the correlation between the covariates and to the limited number of subjects available for the analysis. In case of small populations, incorrect covariates might be selected due to lack of balance of the covariate distribution in the population analysed during model building; the selection of the erroneous covariates could have serious consequences in the prediction of pharmacokinetic or pharmacodynamic parameters in a different population.

Despite some limitations, pharmacokinetic-pharmacodynamic modelling in paediatrics constitutes a powerful and innovative approach to characterise pharmacokinetic-pharmacodynamic relationships and to optimise dosing regimens for children of different ages, body weights and genetic backgrounds. It has been widely applied to antiretroviral therapy to relate plasma concentration to efficacy and to identify the optimal dose of antiretroviral drugs in children (103;104).

Modelling patient adherence

In order to explore novel regimens of antiretroviral drugs or optimise existing ones, a third statistical element needs to be implemented, which describes the patients and their behaviour towards the treatment.

As explained in the previous part of this review, dosing patterns may differ between patients in terms of the actual dose (compliance), the timing of doses (quality) and the duration of treatment (persistence) (105-107). The consequences of variable adherence on treatment outcomes are determined by the magnitude of erratic dosing about the prescribed dosing times, the number and frequency of sequentially missed doses or “drug holidays” (when the patient stops taking the medication(s) for a period of time) and the pharmacological properties of the drug (108). Based on clinical data of adherence to treatment a very large inter-individual variability has been identified in dosing timing relative to the prescribed interdose interval. Indices of dose-taking compliance (the quantity of the dose) are usually less variable (109).

Given the need to replace compliance data when not available or to test the impact of various non-compliance scenarios, several simulation models of treatment execution have been proposed in the past years. The simplest one assumes that the prescribed number of pills was taken correctly, but at different times than the prescribed ones (110;111). In those models, time intervals between two doses are drawn from normal distributions (112). Other models propose to simulate number of doses taken at each dose time according to a multinomial distribution allowing for 0, 1, 2 or more doses taken at each dosing time (113). Since this number may depend on the number of doses previously taken, an earlier attempt suggested using a Markov model (114), which has great flexibility and allows description of almost all different compliance profiles. The use of covariates in this model allows controlling, for example, the date at which the patient will have a “drug holiday”.

In conjunction with variable compliance, patient drop-out constitutes another fundamental element in clinical trials. Two types of drop-out exist: non-informative and informative drop-outs. Non-informative drop-outs simply mean that some patients may randomly stop to be reported in the trial, this independently from the treatment they received, and this independently of efficacy or toxic effects. On the contrary, disease progress can be correlated to the marker that is being followed. In this case, the drop-out is informative to the disease progress, and modelling the disease progress separately from the drop-out process may be inefficient and may produce bias estimates (115;116). For example, in a trial of HIV treatment, disease progress may lead a patient to drop-out to seek other treatment options.

Trial execution models, such as compliance and drop-out models, interact with the drug-disease models as depicted in figure 5.

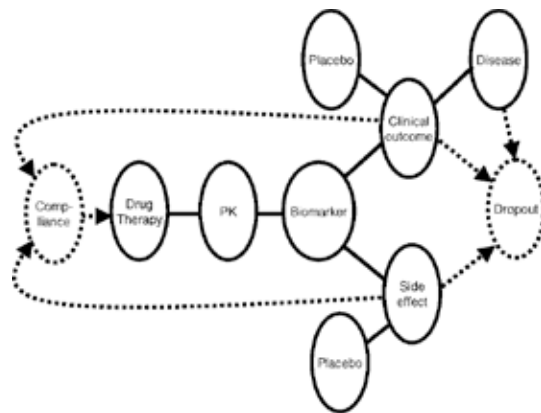


Figure 5 Schematic representation of interactions between drug-disease models and two aspects of execution model: the compliance model and the dropout model. Drug-disease models interactions are shown with continuous lines, while execution models are presented with dotted line arrows. Notice that compliance influences pharmacokinetic and pharmacodynamic models, which in turn, by feedback mechanisms, may influence compliance. (source: <http://www.euroformhealthcare.info/drug-development/protocol-deviations-and-execution-models.html>).

Clinical trial simulation

Given the characteristics of the HIV-infected population, a model-based approach is a potent instrument able to define and characterise the processes driving the disease, the pharmacokinetic and pharmacodynamic properties of the drug and the behaviour of the patient towards the prescribed treatment. Two elements of a model-based approach need to be distinguished and defined: modelling and simulation. The former enables translation of the relevant features of a system into mathematical language (i.e. model parameters), whilst the latter allows the assessment of a system's performance under hypothetical and real-life scenarios (i.e. “what-if” scenarios), yielding information about the implication of different experimental designs and quantitative predictions about treatment outcome, dosing requirements and covariate effects (117;118). In clinical trial simulation (CTS) multiple factors can be evaluated concurrently and relevant scenarios can be defined and investigated. The great advantage of the use of CTS in paediatric drug development and clinical practice is the possibility of exploring relevant scenarios before enrolling children into a clinical protocol (119;120). Simulations allow evaluation of a range of parameter values, including an assessment of critical scenarios, such as overdosing, that cannot be generated in real-life studies (94).

CTS has been widely used in the past in paediatric drug development and clinical practice (121). Lärer *et al.* used CTS to develop an age-specific dosing regimen for sotalol in children (122), a CTS evaluation by Yim *et al.* (123) was used to get US Food and Drug Administration approval to change the dosing regimen for etanercept in juvenile rheumatoid arthritis and CTS was applied to select rufinamide doses giving an exposure shown to be safe and efficacious in large paediatric populations (124). In CTS three important components are characterised: a disease/placebo model, a drug model, and the implementation model (trial design and decision criteria) (fig 6). Together with a model which describes the biological mechanisms underlying the disease (125) and a drug-action model which comprises pharmacokinetic and pharmacodynamic factors (126), a trial model that simulates other important aspects of the trial, such as dropout, compliance and protocol deviations, is required (127). Thus far, despite the widespread use of CTS in paediatrics, very few examples exist in which relevant design factors have been evaluated prospectively as part of the planning of a paediatric trial. In particular patient-related components, such as adherence and drop-out have not been encompassed in previous paediatric CTS.



Figure 6 The diagram depicts the major components of a clinical trial simulation (CTS). In model-based drug development, CTS can be used to characterise the interactions between drug and disease, enabling among other things the assessment of disease-modifying effects, dose selection and covariate effects (e.g. age, body weight). In conjunction with a trial model, CTS allows the evaluation of such interactions, taking into account uncertainty and trial design factors, including the implications of different statistical methods for the analysis of the data.

Using the pharmacokinetic-pharmacodynamic relationships and viral dynamics, clinical trial simulations with antiviral drugs constitutes a very powerful tool to assess the impact of patient-, drug- and protocol-related factors on trial outcome and identify critical factors, such as dose selection, and influence of covariates. CTS may be used to evaluate the consequences of different patterns of adherence on treatment outcome for specific drugs or drug combinations, yielding a quantitative and systematic definition of forgiveness of non-adherence for each antiretroviral drug. Such information could be of indisputable value in the exploration of situations which have not been tested in reality, such as new doses, new dosing regimens or new drug combinations.

In the previous paragraphs the advantages of model-based approaches for the characterisation of pharmacokinetics and pharmacodynamics in children have been extensively elucidated. However a previous investigation has shown that limitations exist in such approaches when extrapolations are required from different paediatric populations (128). The use of parametric approaches must take into account that parameter estimation uncertainty is often accompanied by model uncertainty and eventual misspecifications. Therefore the use of adaptive protocols in the settings of clinical trial simulation which include monitoring of clinical endpoints at pre-defined intervals may be considered to overcome uncertainty in parameter distributions and obtain better dosing recommendations in children (129).

1.3. CONCLUSIVE REMARKS

The previous paragraphs highlighted three main issues in antiretroviral paediatric therapy: the selection of the dose, the optimisation of the dosing regimen and the problem of adherence to treatment. The use of an integrated model-based approach in which pharmacokinetic-pharmacodynamic relationships, viral dynamics, patient behaviour and trial execution factors are incorporated provides the basis for comprehensive clinical trial simulation scenarios. CTS represents a critical step in the evaluation and planning of experimental protocols. It also offers the opportunity to explore conditions which may not be feasible or ethically acceptable in children. The possibility to evaluate *in silico* populations of HIV-infected children without exposing the patients to experimental settings will strongly simplify the identification of the best dose or dosing regimen for a selected group of HIV-infected children and the investigation of forgiveness of non-adherence of current or future treatments.

References

1. Hazra R, Siberry GK, Mofenson LM. Growing up with HIV: children, adolescents, and young adults with perinatally acquired HIV infection. *Annual review of medicine* **61**, 169-85 (2010).
2. Polisset J, Ametonou F, Arrive E, Aho A, Perez F. Correlates of adherence to antiretroviral therapy in HIV-infected children in Lome, Togo, West Africa. *AIDS behavior* **13**(1), 23-32 (2009).
3. George E, Noel F, Bois G, Cassagnol R, Estavien L, Rouzier PM et al. Antiretroviral therapy for HIV-1-infected children in Haiti. *Journal of infectious diseases* **195**(10), 1411-8 (2007).
4. Castro H, Judd A, Gibb DM, Butler K, Lodwick RK, van SA et al. Risk of triple-class virological failure in children with HIV: a retrospective cohort study. *Lancet* **377**, 1580-7 (2011).
5. Mukhtar M., Arshad M., Ahmad M., Pomerantz R.J, Wigdahl B., Parveen Z. Antiviral potentials of medicinal plants. *Virus Research* **131**(2):111-20 (2008)
6. Cortez KJ, Maldarelli F. Clinical Management of HIV Drug Resistance. *Viruses* **3**(4), 347-78 (2011)
7. Paredes R, Clotet B. Clinical management of HIV-1 resistance. *Antiviral research* **85**(1), 245-65 (2010).
8. Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* **271**(5255), 1582-6 (1996).
9. Volberding PA, Deeks SG. Antiretroviral therapy and management of HIV infection. *Lancet* **376**(9734), 49-62 (2010).
10. Garnett GP, Bartley L, Grassly NC, Anderson RM. Antiretroviral therapy to treat and prevent HIV/AIDS in resource-poor settings. *Nature medicine* **8**(7), 651-4 (2002).
11. Grant RM, Kuritzkes DR, Johnson VA, Mellors JW, Sullivan JL, Swanstrom R et al. Accuracy of the TRUGENE HIV-1 genotyping kit. *Journal of clinical microbiology* **41**(4), 1586-93 (2003).
12. Pandit A, Mackay WG, Steel C, van Loon AM, Schuurman R. HIV-1 drug resistance genotyping quality assessment: results of the ENVA7 Genotyping Proficiency Programme. *Journal of clinical virology* **43**(4), 401-6 (2008).
13. Antiretroviral therapy for HIV infection in infants and children. Available at <http://www.who.int/hiv/pub/paediatric/paed-prelim-summary.pdf> (last accessed 24 April 2013)
14. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Available at: <http://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-treatment-guidelines/0> (last accessed 24 April 2013)
15. Ruane PJ, Richmond GJ, Dejesus E, Hill-Zabala CE, Danehower SC, Liao Q et al. Pharmacodynamic effects of zidovudine 600 mg once/day versus 300 mg twice/day in therapy-naive patients infected with human immunodeficiency virus. *Pharmacotherapy* **24**(3), 307-12 (2004).
16. Tudor-Williams G, St Clair MH, McKinney RE, Maha M, Walter E, Santacroce S et al. HIV-1 sensitivity to zidovudine and clinical outcome in children. *Lancet* **339**(8784), 15-9 (1992)
17. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. Available at: <http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf> (last accessed 24 April 2013)
18. Johnson MA, Moore KH, Yuen GJ, Bye A, Pakes GE. Clinical pharmacokinetics of lamivudine. *Clinical pharmacokinetics* **36**(1), 41-66 (1999).
19. Selected Properties of Lamivudine. Available at: http://www.hivclinic.ca/main/drugs_properties_files/RTI%20lamivudine.pdf (last accessed 24 April 2013)
20. Yuen GJ, Weller S, Pakes GE. A review of the pharmacokinetics of abacavir. *Clinical pharmacokinetics* **47**(6), 351-71 (2008).
21. Melroy J, Nair V. The antiviral activity, mechanism of action, clinical significance and resistance of abacavir in the treatment of pediatric AIDS. *Current pharmaceutical design* **11**(29), 3847-52 (2005).
22. Hirt D, Bardin C, Diagbouga S, Nacro B, Hien H, Zoure E et al. Didanosine population pharmacokinetics in West African human immunodeficiency virus-infected children administered once-daily tablets in relation to efficacy after one year of treatment. *Antimicrobial agents and chemotherapy* **53**(10), 4399-406 (2009).
23. Selected Properties of Didanosine. Available at: http://www.hivclinic.ca/main/drugs_properties_files/RTI_didanosine.pdf (last accessed 24 April 2013)

24. Hemanth Kumar AK, Ramachandran G, Rajasekaran S, Padmapriyadarsini C, Narendran G, Anitha S et al. Pharmacokinetics of lamivudine & stavudine in generic fixed-dose combinations in HIV-1 infected adults in India. *Indian journal of medical research* **130**(4), 451-7 (2009).
25. Riddler SA, Anderson RE, Mellors JW. Antiretroviral activity of stavudine (2',3'-didehydro-3'-deoxythymidine, D4T). *Antiviral research* **27**(3), 189-203 (1995).
26. Molina JM. Emtricitabine: a new treatment option for simplified antiretroviral therapy. *AIDS Read* S16-S21 (2003).
27. Hazra R, Balis FM, Tullio AN, DeCarlo E, Worrell CJ, Steinberg SM et al. Single-dose and steady-state pharmacokinetics of tenofovir disoproxil fumarate in human immunodeficiency virus-infected children. *Antimicrobial agents and chemotherapy* **48**(1), 124-9 (2004).
28. Selected Properties of Tenofovir. Available at: http://www.hivclinic.ca/main/drugs_properties_files/RTI%20tenofovir.pdf (last accessed 24 April 2013)
29. Ribaud HJ, Haas DW, Tierney C, Kim RB, Wilkinson GR, Gulick RM et al. Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study. *Clinical infectious diseases* **42**(3), 401-7 (2006).
30. Best BM, Koopmans PP, Letendre SL, Capparelli EV, Rossi SS, Clifford DB et al. Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. *Journal of antimicrobial chemotherapy* **66**(2), 354-7 (2011).
31. Selected Properties of Nevirapine. Available at: http://www.hivclinic.ca/main/drugs_properties_files/NNRTI%20nevirapine.pdf (last accessed 24 April 2013)
32. Cvetkovic RS, Goa KL. Lopinavir/ritonavir: a review of its use in the management of HIV infection. *Drugs* **63**(8), 769-802 (2003).
33. Smith PF, Robbins GK, Shafer RW, Wu H, Yu S, Hirsch MS et al. Pharmacokinetics of nelfinavir and efavirenz in antiretroviral-naïve, human immunodeficiency virus-infected subjects when administered alone or in combination with nucleoside analog reverse transcriptase inhibitors. *Antimicrobial agents and chemotherapy* **49**(8), 3558-61 (2005).
34. Rodes B, Sheldon J, Toro C, Jimenez V, Alvarez MA, Soriano V. Susceptibility to protease inhibitors in HIV-2 primary isolates from patients failing antiretroviral therapy. *Journal of antimicrobial chemotherapy* **57**(4), 709-13 (2006).
35. Boffito M, Else L, Back D, Taylor J, Khoo S, Sousa M et al. Pharmacokinetics of atazanavir/ritonavir once daily and lopinavir/ritonavir twice and once daily over 72 h following drug cessation. *Antiviral therapy* **13**(7), 901-7 (2008).
36. Selected Properties of Atazanavir. Available at: http://www.hivclinic.ca/main/drugs_properties_files/PI%20atazanavir.pdf (last accessed 24 April 2013)
37. Cella M, Knibbe C, Danhof M, Della PO. What is the right dose for children? *British journal of clinical pharmacology* **70**(4), 597-603 (2010).
38. Rodriguez W, Selen A, Avant D, Chaurasia C, Crescenzi T, Gieser G et al. Improving pediatric dosing through pediatric initiatives: what we have learned. *Pediatrics* **121**(3), 530-9 (2008).
39. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *New England journal of medicine* **349**(12), 1157-67 (2003).
40. Kearns GL, Reed MD. Clinical pharmacokinetics in infants and children. A reappraisal. *Clinical pharmacokinetics* **17** Suppl 1, 29-67 (1989).
41. Neely MN, Rakhmanina NY. Pharmacokinetic optimization of antiretroviral therapy in children and adolescents. *Clinical pharmacokinetics* **50**(3), 143-89 (2011).
42. Mahmood I. Prediction of drug clearance in children: impact of allometric exponents, body weight, and age. *Therapeutic drug monitoring* **29**(3), 271-8 (2007).
43. van Onzenoort HA, Menger FE, Neef C, Verberk WJ, Kroon AA, de Leeuw PW et al. Participation in a clinical trial enhances adherence and persistence to treatment: a retrospective cohort study. *Hypertension* **58**(4), 573-8 (2011).
44. Van Dyke RB, Lee S, Johnson GM, Wiznia A, Mohan K, Stanley K et al. Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. *Pediatrics* **109**(4), e61 (2002).
45. Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Annals of internal medicine* **133**(1), 21-30 (2000).

46. Vrijens B, Goetghebeur E, de KE, Rode R, Mayer S, Urquhart J. Modelling the association between adherence and viral load in HIV-infected patients. *Statistics in medicine* **24**(17), 2719-31 (2005).
47. Vreeman RC, Wiehe SE, Pearce EC, Nyandiko WM. A systematic review of pediatric adherence to antiretroviral therapy in low- and middle-income countries. *Pediatric infectious diseases J* **27**(8), 686-91 (2008).
48. Vrijens B, Urquhart J. Patient adherence to prescribed antimicrobial drug dosing regimens. *Journal of antimicrobial chemotherapy* **55**(5), 616-27 (2005).
49. Hano T. Improvement of adherence in the treatment of hypertension. *Nihon Rinsho* **69**(11), 1982-6 (2011).
50. Sleath B, Blalock S, Covert D, Stone JL, Skinner AC, Muir K et al. The Relationship between Glaucoma Medication Adherence, Eye Drop Technique, and Visual Field Defect Severity. *Ophthalmology* **118**(12), 2398-402 (2011).
51. Cate H, Broadway DC. Association between intraocular pressure and adherence: is there one? *Eye (Lond)* **25**(9), 1238-9 (2011).
52. Sampalis JS, Adachi JD, Rampakakis E, Vaillancourt J, Karellis A, Kindundu C. Long-term impact of adherence to oral bisphosphonates on osteoporotic fracture incidence. *Journal of bone and mineral research* **27** (1), 202-10 (2011).
53. Burack G, Gaur S, Marone R, Petrova A. Adherence to antiretroviral therapy in pediatric patients with human immunodeficiency virus (HIV-1). *Journal of pediatric nursing* **25**(6), 500-4 (2010).
54. Jaspan HB, Mueller AD, Myer L, Bekker LG, Orrell C. Effect of caregivers' depression and alcohol use on child antiretroviral adherence in South Africa. *AIDS patient care STDS* **25**(10), 595-600. (2011).
55. Skovdal M, Campbell C, Madanhire C, Nyamukapa C, Gregson S. Challenges faced by elderly guardians in sustaining the adherence to antiretroviral therapy in HIV-infected children in Zimbabwe. *AIDS Care* **23**(8), 957-64 (2011).
56. Malee K, Williams P, Montepiedra G, McCabe M, Nichols S, Sirois PA et al. Medication adherence in children and adolescents with HIV infection: associations with behavioral impairment. *AIDS patient care STDS* **25**(3), 191-200 (2011).
57. Biadgilign S, Deribew A, Amberbir A, Deribe K. Barriers and facilitators to antiretroviral medication adherence among HIV-infected paediatric patients in Ethiopia: A qualitative study. *SAHARA J* **6**(4), 148-54 (2009).
58. Paranthaman K, Kumarasamy N, Bella D, Webster P. Factors influencing adherence to anti-retroviral treatment in children with human immunodeficiency virus in South India--a qualitative study. *AIDS Care* **21**(8), 1025-31 (2009).
59. Nicca D, Moody K, Elzi L, Spirig R. Comprehensive clinical adherence interventions to enable antiretroviral therapy: a case report. *Journal of the association of nurses in AIDS care* **18**(6), 44-53 (2007).
60. McPherson-Baker S, Jones D, Duran RE, Klimas N, Schneiderman N. Development and implementation of a medication adherence training instrument for persons living with HIV: the MATI. *Behavior modification* **29**(2), 286-317 (2005).
61. Coetzee D, Boule A, Hildebrand K, Asselman V, Van CG, Goemaere E. Promoting adherence to antiretroviral therapy: the experience from a primary care setting in Khayelitsha, South Africa. *AIDS* **18**, S27-S31 (2004).
62. Saini SD, Schoenfeld P, Kaulback K, Dubinsky MC. Effect of medication dosing frequency on adherence in chronic diseases. *American journal of managed care* **15**(6), e22-e33 (2009).
63. Hawkins T. Impact of once- and twice-daily dosing regimens on adherence and overall safety. *AIDS Read* **14**(6), 320-31, 334 (2004).
64. Leenen FH, Wilson TW, Bolli P, Larochelle P, Myers M, Handa SP et al. Patterns of compliance with once versus twice daily antihypertensive drug therapy in primary care: a randomized clinical trial using electronic monitoring. *Canadian journal of cardiology* **13**(10), 914-20 (1997).
65. Eron JJ, Feinberg J, Kessler HA, Horowitz HW, Witt MD, Carpio FF et al. Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naïve HIV-positive patients: a 48-week randomized clinical trial. *Journal of infectious diseases* **189**(2), 265-72 (2004).
66. Boffito M, Dickinson L, Hill A, Back D, Moyle G, Nelson M et al. Pharmacokinetics of once-daily saquinavir/ritonavir in HIV-infected subjects: comparison with the standard twice-daily regimen. *Antiviral therapy* **9**(3), 423-9. (2004).

67. Comte L, Vrijens B, Tousset E, Gerard P, Urquhart J. Estimation of the comparative therapeutic superiority of QD and BID dosing regimens, based on integrated analysis of dosing history data and pharmacokinetics. *Journal of pharmacokinetics and pharmacodynamics* **34**(4):549-58 (2007).
68. Dickinson L, Boffito M, Khoo SH, Schutz M, Aarons LJ, Pozniak AL et al. Pharmacokinetic analysis to assess forgiveness of boosted saquinavir regimens for missed or late dosing. *Journal of antimicrobial chemotherapy* **62**(1), 161-7 (2008).
69. Bangsberg DR, Moss AR, Deeks SG. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. *Journal of antimicrobial chemotherapy* **53**(5), 696-9 (2004).
70. Bangsberg DR, Acosta EP, Gupta R, Guzman D, Riley ED, Harrigan PR et al. Adherence-resistance relationships for protease and non-nucleoside reverse transcriptase inhibitors explained by virological fitness. *AIDS* **20**(2), 223-31 (2006).
71. Shuter J. Forgiveness of non-adherence to HIV-1 antiretroviral therapy. *Journal of antimicrobial chemotherapy* **61**(4), 769-73 (2008).
72. Kenna LA, Labbe L, Barrett JS, Pfister M. Modeling and simulation of adherence: approaches and applications in therapeutics. *AAPS Journal* **7**(2), E390-E407 (2005).
73. Hughes DA, Walley T. Predicting "real world" effectiveness by integrating adherence with pharmacodynamic modeling. *Clinical pharmacology and therapeutics* **74**(1), 1-8 (2003).
74. Johnson BF, Whelton A. A Study Design for Comparing the Effects of Missing Daily Doses of Antihypertensive Drugs. *American journal of therapeutics* **1**(4), 260-7 (1994).
75. Vaur L, Dutrey-Dupagne C, Boussac J, Genes N, Bouvier dM, Elkik F et al. Differential effects of a missed dose of trandolapril and enalapril on blood pressure control in hypertensive patients. *Journal of cardiovascular pharmacology* **26**(1), 127-31 (1995).
76. Bellanti F, Della PO. Modelling and simulation as research tools in paediatric drug development. *European journal of clinical pharmacology* **67** Suppl 1, 75-86 (2011).
77. Layden TJ, Layden JE, Ribeiro RM, Perelson AS. Mathematical modeling of viral kinetics: a tool to understand and optimize therapy. *Clinical liver disease* **7**(1), 163-78 (2003).
78. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* **373**(6510), 117-22 (1995).
79. Nowak MA, Lloyd AL, Vasquez GM, Wiltout TA, Wahl LM, Bischofberger N et al. Viral dynamics of primary viremia and antiretroviral therapy in simian immunodeficiency virus infection. *Journal of virology* **71**(10):7518-25 (1997).
80. Bonhoeffer S, May RM, Shaw GM, Nowak MA. Virus dynamics and drug therapy. *Proceedings of the national academy of sciences U S A* **94**(13), 6971-6 (1997).
81. Perelson AS. Modelling viral and immune system dynamics. *Nature reviews immunology* **2**(1), 28-36 (2002).
82. Nelson PW, Perelson AS. Mathematical analysis of delay differential equation models of HIV-1 infection. *Mathematical biosciences* **179**(1):73-94 (2002).
83. Wein LM, D'Amato RM, Perelson AS. Mathematical analysis of antiretroviral therapy aimed at HIV-1 eradication or maintenance of low viral loads. *Journal of theoretical biology* **192**(1):81-98 (1998).
84. Ding AA, Wu H. Relationships between antiviral treatment effects and biphasic viral decay rates in modeling HIV dynamics. *Mathematical biosciences* **160**(1), 63-82 (1999).
85. Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K et al. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* **387**(6629), 188-91 (1997).
86. Funk GA, Fischer M, Joos B, Opravil M, Gunthard HF, Ledergerber B et al. Quantification of in vivo replicative capacity of HIV-1 in different compartments of infected cells. *Journal of acquired immune deficiency syndrome* **26**(5), 397-404 (2001).
87. Wu H, Huang Y, Acosta EP, Rosenkranz SL, Kuritzkes DR, Eron JJ et al. Modeling long-term HIV dynamics and antiretroviral response: effects of drug potency, pharmacokinetics, adherence, and drug resistance. *Journal of acquired immune deficiency syndrome* **39**(3), 272-83 (2005).
88. Banks HT, Davidian M, Hu S, Kepler GM, Rosenberg ES. Modeling HIV Immune Response and Validation with Clinical Data. *Journal of biological dynamics* **2**(4), 357-85 (2008).
89. Rosenberg ES, Davidian M, Banks HT. Using mathematical modeling and control to develop structured treatment interruption strategies for HIV infection. *Drug alcohol dependence* **88** Suppl 2, S41-S51 (2007).

90. Wu H, Huang Y, Acosta EP, Park JG, Yu S, Rosenkranz SL et al. Pharmacodynamics of antiretroviral agents in HIV-1 infected patients: using viral dynamic models that incorporate drug susceptibility and adherence. *Journal of pharmacokinetics and pharmacodynamics* **33**(4), 399-419 (2006).
91. Rosario MC, Jacqmin P, Dorr P, van der Ryst E, Hitchcock C. A pharmacokinetic-pharmacodynamic disease model to predict in vivo antiviral activity of maraviroc. *Clinical pharmacology and therapeutics* **78**(5), 508-19 (2005).
92. De Cock RF, Piana C, Krekels EH, Danhof M, Allegaert K, Knibbe CA. The role of population PK-PD modelling in paediatric clinical research. *European journal of clinical pharmacology* **67** Suppl 1, 5-16 (2011).
93. Wright DF, Winter HR, Duffull SB. Understanding the time course of pharmacological effect: a PKPD approach. *British journal of clinical pharmacology* **71**(6), 815-23 (2011).
94. Anderson BJ, Allegaert K, Holford NH. Population clinical pharmacology of children: general principles. *European journal of pediatrics* **165**(11), 741-6 (2006).
95. Wade JR, Beal SL, Sambol NC. Interaction between structural, statistical, and covariate models in population pharmacokinetic analysis. *Journal of pharmacokinetic and biopharmaceutics* **22**(2), 165-77 (1994).
96. Anderson BJ, Allegaert K, Holford NH. Population clinical pharmacology of children: modelling covariate effects. *European journal of pediatrics* **165**(12), 819-29 (2006).
97. Knibbe CA, Danhof M. Individualized dosing regimens in children based on population PKPD modelling: are we ready for it? *International journal of pharmaceutics* **415**(1-2), 9-14 (2011).
98. Anderson BJ, Holford NH. Mechanistic basis of using body size and maturation to predict clearance in humans. *Drug metabolism and pharmacokinetics* **24**(1), 25-36 (2009).
99. Meibohm B, Laer S, Panetta JC, Barrett JS. Population pharmacokinetic studies in pediatrics: issues in design and analysis. *AAPS Journal* **7**(2), E475-E487 (2005).
100. Mahmood I. Application of fixed exponent 0.75 to the prediction of human drug clearance: an inaccurate and misleading concept. *Drug metabolism and drug interactions* **24**(1), 57-81 (2009).
101. Knibbe CA, Krekels EH, van den Anker JN, DeJongh J, Santen GW, van DM et al. Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. *Clinical pharmacokinetics* **48**(6), 371-85 (2009).
102. Sheiner LB, Ludden TM. Population pharmacokinetics/dynamics. *Annual review of pharmacology and toxicology* **32**, 185-209 (1992).
103. Dumon C, Solas C, Thuret I, Chambost H, Lacarelle B, Michel G et al. Relationship between efficacy, tolerance, and plasma drug concentration of ritonavir in children with advanced HIV infection. *Therapeutic drug monitoring* **22**(4), 402-8 (2000).
104. Crawford KW, Li C, Keung A, Su Z, Hughes MD, Greaves W et al. Pharmacokinetic/pharmacodynamic modeling of the antiretroviral activity of the CCR5 antagonist Vicriviroc in treatment experienced HIV-infected subjects (ACTG protocol 5211). *Journal of acquired immune deficiency syndrome* **53**(5), 598-605 (2010).
105. Urquhart J. The electronic medication event monitor. Lessons for pharmacotherapy. *Clinical pharmacokinetics* **32**(5), 345-56 (1997).
106. Urquhart J. Ascertaining how much compliance is enough with outpatient antibiotic regimens. *Postgraduate medical journal* **68** Suppl 3, S49-S58 (1992).
107. Urquhart J, de KE. Contending paradigms for the interpretation of data on patient compliance with therapeutic drug regimens. *Statistics in medicine* **17**(3), 251-67 (1998).
108. Kastrissios H, Suarez JR, Katzenstein D, Girard P, Sheiner LB, Blaschke TF. Characterizing patterns of drug-taking behavior with a multiple drug regimen in an AIDS clinical trial. *AIDS* **12**(17), 2295-303 (1998).
109. Kastrissios H, Suarez JR, Hammer S, Katzenstein D, Blaschke TF. The extent of non-adherence in a large AIDS clinical trial using plasma dideoxynucleoside concentrations as a marker. *AIDS* **12**(17), 2305-11 (1998).
110. Nony P, Cucherat M, Boissel JP. Revisiting the effect compartment through timing errors in drug administration. *Trends in pharmacological sciences* **19**(2), 49-54 (1998).
111. Vrijens B, Goetghebeur E. The impact of compliance in pharmacokinetic studies. *Statistical methods in medical research* **8**(3), 247-62 (1999).

112. Wang W, Husan F, Chow SC. The impact of patient compliance on drug concentration profile in multiple doses. *Statistics in medicine* **15**(6), 659-69 (1996).
113. Wang W, Ouyang SP. The formulation of the principle of superposition in the presence of non-compliance and its applications in multiple dose pharmacokinetics. *Journal of pharmacokinetics and biopharmaceutics* **26**(4), 457-69 (1998).
114. Girard P, Blaschke TF, Kastrissios H, Sheiner LB. A Markov mixed effect regression model for drug compliance. *Statistics in medicine* **17**(20), 2313-33 (1998).
115. Jonsson EN, Sheiner LB. More efficient clinical trials through use of scientific model-based statistical tests. *Clinical pharmacology and therapeutics* **72**(6), 603-14 (2002).
116. Hu C, Sale ME. A joint model for nonlinear longitudinal data with informative dropout. *Journal of pharmacokinetics and pharmacodynamics* **30**(1), 83-103 (2003).
117. Manolis E, Pons G. Proposals for model-based paediatric medicinal development within the current European Union regulatory framework. *British journal of clinical pharmacology* **68**(4), 493-501 (2009).
118. Laer S, Barrett JS, Meibohm B. The in silico child: using simulation to guide pediatric drug development and manage pediatric pharmacotherapy. *Journal of clinical pharmacology* **49**(8), 889-904 (2009).
119. Girard P, Cucherat M, Guez D. Clinical trial simulation in drug development. *Therapie* **59**(3), 287-304 (2004).
120. Girard P. Clinical trial simulation: a tool for understanding study failures and preventing them. *Basic and clinical pharmacology and toxicology* **96**(3), 228-34 (2005).
121. Holford N, Ma SC, Ploeger BA. Clinical trial simulation: a review. *Clinical pharmacology and therapeutics* **88**(2), 166-82 (2010).
122. Laer S, Elshoff JP, Meibohm B, Weil J, Mir TS, Zhang W et al. Development of a safe and effective pediatric dosing regimen for sotalol based on population pharmacokinetics and pharmacodynamics in children with supraventricular tachycardia. *Journal of the American college of cardiology* **46**(7):1322-30 (2005).
123. Yim DS, Zhou H, Buckwalter M, Nestorov I, Peck CC, Lee H. Population pharmacokinetic analysis and simulation of the time-concentration profile of etanercept in pediatric patients with juvenile rheumatoid arthritis. *Journal of clinical pharmacology* **45**(3), 246-56 (2005).
124. Marchand M, Fuseau E, Critchley DJ. Supporting the recommended paediatric dosing regimen for rifinamide in Lennox-Gastaut syndrome using clinical trial simulation. *Journal of pharmacokinetics and pharmacodynamics* **37**(1), 99-118 (2010).
125. Bies RR, Gastonguay MR, Schwartz SL. Mathematics for understanding disease. *Clinical pharmacology and therapeutics* **83**(6):904-8 (2008).
126. Santen G, van ZE, Danhof M, Della PO. From trial and error to trial simulation. Part 1: the importance of model-based drug development for antidepressant drugs. *Clinical pharmacology and therapeutics* **86**(3), 248-54 (2009).
127. Gobburu JV, Lesko LJ. Quantitative disease, drug, and trial models. *Annual Review of pharmacology and toxicology* **49**, 291-301 (2009).
128. Cella M, Zhao W, Jacqz-Aigrain E, Burger D, Danhof M, Della PO. Paediatric drug development: are population models predictive of pharmacokinetics across paediatric populations? *British journal of clinical pharmacology* **72**(3), 454-64 (2011).
129. Cella M, Danhof M, Della PO. Adaptive trials in paediatric development: dealing with heterogeneity and uncertainty in pharmacokinetic differences in children. *British journal of clinical pharmacology* **74** (2), 346-53 (2012).

SCOPE AND INTENT OF THE INVESTIGATION

Undoubtedly, considerable advancements have been made in the last decades in terms of efficacy and availability of treatment options for HIV. Cocktails containing three or more antiretroviral drugs are now available, as well as fixed-dose combinations which may significantly improve patient's quality of life. However, there are other issues that are still unresolved when dealing with paediatric HIV. Despite the abovementioned advancements, an estimated 2.5 million children worldwide are infected with HIV; 90% of them live in sub-Saharan Africa (SSA), where annually 330000 new infections occur (1). HIV remains a disease widely spread in limited-resources areas where the access to the medication is inadequate and monitoring of antiretroviral therapy is challenging if not impossible in clinical practice. In addition, the pill burden for patients and the potential for drug-drug interactions that can compromise the outcome of the treatment continue to be high.

The chronicity of the treatment implies the administration of several drugs throughout childhood. Furthermore, dose adjustment is often necessary to ensure that appropriate exposure is achieved and maintained during the course of therapy. Among other factors, physiological growth and development in the paediatric patients can significantly affect ADME processes, in particular drug absorption and elimination. Immature renal function, altered hepatic enzyme activity and differences in drug absorption lead to variations in the systemic exposure of antiretroviral drugs among children (2).

From a therapeutic perspective, growing evidence also reveals that treatment adherence in HIV-infected children is complex and current levels are often suboptimal. Poor adherence to therapy may compromise the outcome of the treatment, even when the administered dose takes into account demographic characteristics and growth-related changes. It can lead to the development of drug resistance towards most medications, which ultimately results in viral failure. Adherence is influenced by many factors, which may be categorised as characteristics of the child, the caregiver(s) and family, the formulation and the regimen (3). Paediatric patients usually rely on a caregiver to receive their medication, which may be a challenge when frequent dosing is required. In this context, a reduction in dosing frequency has been considered critical for the optimisation of antiretroviral therapy. Yet, one should bear in mind that simplified

dosing regimens should not increase the risk of under or over exposure. Such an evaluation is complicated by the fact that efficacy and safety in patients must be assessed for combination therapies, in which the contribution of each single drug is often not well defined.

Based on the aforementioned, it is hard to understand why the choice of the dose and dosing regimens for the treatment of paediatric HIV continues to be driven by empirical decisions. An integrated approach is required in which the evaluation of efficacy and safety is driven not only by the evidence arising from paediatric clinical trials, but also by inferences from historical data and quantitative pharmacology concepts.

The aim of this thesis is therefore to explore opportunities to support evidence generation and extrapolation across populations, with special focus on the selection of the dose and dosing regimens in HIV-infected children. Here we propose the use of a model-based approach to identify and quantify the potential causes of variation in drug pharmacokinetics in children, taking into account the interaction between patient adherence and the pharmacokinetic-pharmacodynamic properties of antiretroviral drugs. Furthermore, given the known challenges in running clinical trials in children, we will also show the utility of clinical trial simulations and role of simulation scenarios to assess the implications of changes in pharmacokinetics and pharmacodynamics associated with different doses, dosing regimens and variable adherence patterns, which cannot be controlled or evaluated in an ethically acceptable manner in clinical practice.

Three central questions will form the basis for the work to be presented in the subsequent chapters of this thesis:

1. How to select the appropriate dose(s) for children given that changes in exposure due to developmental growth are often nonlinearly correlated with body size and evidence from clinical trials is limited to small, imbalanced cohorts?
2. Can changes in dosing regimen be assessed by a model-based approach taking into account the concepts of pharmacokinetic and pharmacokinetic-pharmacodynamic bridging?
3. Can pharmacokinetic-pharmacodynamic relationships be used to evaluate the impact of variable patterns of adherence to therapy on treatment outcome in a quantitative manner, given that this cannot be formally tested in clinical practice due to ethical constraints?

It is our endeavour to show how the use of a model-based approach offers an opportunity to assess the impact of pharmacokinetic differences on pharmacodynamics and consequently enables prediction of treatment outcome (i.e., viral failure) in children before exposing them to a clinical protocol. Ultimately, we will demonstrate that the assumption of similar exposure-response relationships between adult and children enables one not only to account for differences in physiological processes due to developmental growth when selecting the dose, but also provides the basis for evaluating the impact of changes in dosing regimen.

2.1. GENERAL INTRODUCTION

In **Chapter 1**, we provide an overview about the current status of antiretroviral (ART) therapy in children. In fact, we examine the three main elements which undeniably contribute to the outcome of the treatment: the disease, the drug and the patient. First, we explore the concept of drug resistance, the need for drug combination therapy in HIV and the progress achieved in terms of novel interventions, which prevented the development of treatment resistance in the last decades. In addition we introduce the use of mathematical modelling as a tool for characterising infection and viral dynamics in humans. In a subsequent section, we focus on the relevance of statistical population models to characterise the pharmacokinetics and the pharmacodynamics of antiretroviral drugs in children and to quantify the effect of developmental factors on drug exposure and efficacy. Of particular interest is the possibility to apply model-based methodologies to describe and discriminate different sources of variability in pharmacokinetic and pharmacodynamic parameters even when only sparse samples are available. Lastly, we look at the role of the patient in antiretroviral therapy. Different methods are presented, which enable the evaluation of adherence to treatment and how this information can be used to explore variability in treatment outcome. Lastly, we introduce the concept of clinical trial simulations as a tool that allows scrutiny of a variety of factors associated with the drug, the disease, the patient population and the clinical study design prior to enrolment of actual patients into a clinical protocol. Of interest is the possibility to investigate the implications of dose adjustment, titration algorithms and changes in dosing regimen under different scenarios taking treatment adherence into account, rather than considering as a random effect.

Based on the review of the requirements for evidence synthesis in paediatric research, specific issues have been identified in the accepted strategy for the evaluation of the dose and dosing regimens in HIV-infected children which will underpin the scope and intent of the investigations described here in Chapter 2 and detailed in the subsequent sections of this thesis. From a methodological perspective we highlight the need for accurate parameter estimation, the identification of differences in parameter distributions and formal evaluation of the performance of pharmacokinetic-pharmacodynamic models when scaling of the dose and dosing regimens across populations. These principles are then used to support the rationale for extrapolating efficacy across dosing regimens, an approach which is currently not possible according to existing regulatory guidelines. Our work is then extended to include uncertainty and patient-related factors in pharmacokinetic-pharmacodynamic bridging, providing a framework for inferential analysis and evidence synthesis in paediatric drug development.

2.2. SAMPLE SIZE, COVARIATE DISTRIBUTION AND PREDICTIVE PERFORMANCE OF PHARMACOKINETIC MODELS

Pharmacokinetic and pharmacokinetic-pharmacodynamic models must be predictive to be used for the purposes of bridging and extrapolation (4). Therefore uncertainty and bias in parameter estimates need to be assessed accordingly to ensure potential limitations minimised. The predictive performance of a model implies its ability to accurately describe the effects of developmental growth, physiological function and disease across the populations of interest. In this context, the use of small sample sizes has been assumed to be acceptable for data extrapolation, which entails that differences in pharmacokinetics and/or pharmacodynamics are primarily driven by the magnitude of parameter estimates, rather than by distinct structural components (fixed effects) determining drug disposition across populations. This assumption also imposes that a common relationship between parameter and covariates must hold across the various age groups and most likely that common biological substrates are involved from birth throughout to adolescence. Clearly, these considerations may not always be plausible. In fact, available data reveal that pharmacokinetic variability may be caused by different factors at different stages of life (4).

To overcome the potential for bias in the inferences from a pharmacokinetic or pharmacokinetic-pharmacodynamic model, attention must be given to the identification of the mechanisms (and influential factors) underlying differences across populations. Most importantly, one needs to consider which model parameterisation will support accurate dose selection when using models for extrapolation purposes.

It has been previously shown that small samples may increase the probability to introduce bias in the selection of the correct covariate during a stepwise covariate selection (5), which in turns leads to erroneous dosing recommendations. Therefore, in **Chapter 3**, we investigate the impact of sample size on parameter uncertainty and model parameterisation, emphasising the importance of identifying the causes of variability and quantifying their relative contribution (i.e., covariate effect) across populations prior to any inferential analysis. From a methodological perspective, we scrutinise the use of small populations when performing covariate analyses, a common practice which is often justified by ethical and practical limitations in paediatric research. The impact of imbalanced samples across a wide distribution of occurring values and co-linearity between covariates (e.g., age, body weight and height) are investigated.

We perform a meta-analysis in which pharmacokinetic data from three small clinical trials in children aged between 3 months and 12 year old receiving lamivudine are pooled, with a total of 77 children available after combining the three studies. A population pharmacokinetic model is developed taking into account the imbalanced data distribution of reduced sample size on covariate selection. To illustrate the clinical implications of bias in covariate selection, a separate analysis is performed using a subset of the population and the results are subsequently com-

pared with the findings of the meta-analysis. Here we also take the opportunity to demonstrate the importance of comprehensive validation procedures to assess the predictive performance of a model. Such procedures are often omitted in the reporting of population pharmacokinetic and /or pharmacodynamic models (6). We show that a shift in paradigm is needed to allow the use of nonlinear mixed effects modelling not only as a statistical data analysis method, but rather as an inferential tool. In addition, we highlight that the availability of parameter estimates does not automatically translate into dosing regimen recommendations. This represents one of the main shortcomings of model-based analysis of paediatric data. Without comprehensive use of simulations in which variables of interest rather than model parameters are taken into account, one cannot select the correct dose for children and concomitantly assess whether a proposed dosing regimen meets extrapolation or bridging requirements. Given that the main application of models should be the prediction of drug exposure and/or efficacy across different populations, we apply a range of diagnostic tools to explore model performance, including visual predictive check, posterior predictive check, bootstrap, mirror plots and normalised prediction distribution error (NPDE).

Despite the availability of population pharmacokinetic models for abacavir in the published literature, in **Chapter 4** we apply the same meta-analytical approach to this nucleoside reverse transcriptase inhibitor (NRTI) commonly used in combination with lamivudine. In contrast to previous studies, which were based on either a small number of patients or included only a limited, imbalanced sample of the patient population, our analysis shows that accurate characterisation of covariate factors is critical for further assessment of the individual dosing requirements across different age groups. In this context, the use of meta-analysis is proposed as a requirement to ensure that insight is gained into the processes determining maturation and metabolic capacity. Our analysis also shows the potential implication of co-linearity and confounders during covariate model building, which may affect the underlying (true) parameter-covariate correlations. These points are pre-requisites for subsequent use of the model as an inferential tool for predicting drug exposure and/or effect both in individual patients and extrapolation across groups or populations.

Whilst covariate model building and validation procedures are important scientific steps in the implementation of a model-based approach in paediatric drug research, attention should also be paid to the clinical relevance. One should note that the possibility of applying population pharmacokinetic models to predict drug exposure across an age range different from the population used during model building is very appealing, but challenging (7). In fact, evidence from a previous investigation suggests that pharmacokinetic models developed from subsets of the overall population may not be predictive beyond the range of covariates available during model building (8). Given the meta-analytical nature of our approach, in **Chapter 5** we investigate the requirements for the use of simulations in the extrapolation of data across populations. Here we assess the feasibility of extrapolating the variable(s) of interest in a new population

beyond the covariate range explored during model building and the role of covariate-parameter correlations in this process. Different pharmacokinetic models for a hypothetical drug will be used in which the influential demographic factors are linearly or exponentially correlated with clearance. In addition, we explore how the presence of multiple covariate factors affects model parameter estimation and consequently causes bias and confounding with regard to the contribution of each individual. Each model will be used to predict drug exposure in a virtual population of children. Subsequently, two subgroups of children will be identified and the data from the two subgroups will be used to fit a pharmacokinetic model, which in turn will be used to predict drug exposure in the other population. The accuracy and precision of model predictions will be considered as diagnostics of the predictive performance of the model. It will become clear to the readership of this thesis, that the use of simulation tools is crucial to explore the predictive performance of models when investigating the possibility of extrapolating beyond the covariate range explored during model building. This requirement needs to be considered for subsequent application of the models for bridging and extrapolation purposes.

Another methodological issue to be investigated in this chapter is the so-called “centring” on the median or the mean value of the covariate in the population when expressing the covariate-parameter relationship in a model. Thus far, it has been unclear whether the median of the covariate must be kept when the model is used for simulation purposes in a new population or must be adjusted to reflect the covariate distribution in the new population. Again, simulation tools will help us quantifying the accuracy and precision of model predictions when extrapolating across populations.

From a statistical perspective, the modelling issues described here also illustrate the limitations of maximum likelihood methods. Model parameter (fixed and random effects) and covariate selection are determined by diagnostic tools (e.g., goodness-of-fit) which assess model performance relative to the available data, making models primarily descriptive, rather than predictive.

2.3. SIMPLIFIED DOSING REGIMENS IN CHILDREN

In the previous section of this thesis special attention is given to the use of model-based methodologies as a tool for the selection of the appropriate dose(s) for children given that evidence from clinical trials is limited and often derived from small, imbalanced cohorts. Clearly, one needs to account for the effect of developmental growth on pharmacokinetics if the right dose is to be recommended using bridging and extrapolation methods. This also implies accurate identification of the sources of variability and assumptions about the relevance of pharmacokinetic-pharmacodynamic relationships as a proxy for efficacy and safety.

In this context, a second important application of modelling and simulation is the evaluation of changes in dosing regimen. Together with the selection of the optimal dose, dosing frequency

is another challenging aspect in antiretroviral therapy. Reduced dosing frequency, such as once daily administration, may be very appealing for patients and may increase adherence to therapy, as shown previously in various studies (9). Many antiretroviral drugs have been approved in adults for their use as oncedaily dosing. However, fewer options are available in children. In a paediatric population, where adherence can be compromised because of the patients' young age, poor palatability of the medications, and dependence on caregivers, a oncedaily regimen is preferred (10). In this section, we will evaluate the feasibility of oncedaily dosing for lamivudine and abacavir, which are currently approved in adults, but are still recommended according to a twicedaily dosing regimen in children. Despite the favourable pharmacokinetic properties of these drugs and the availability of clinical trials which show comparable pharmacokinetics between once and twice daily dosing, an extensive evaluation in a large paediatric population is required. In **Chapters 6 and 7** the pharmacokinetic models previously developed and validated for lamivudine and abacavir will be used to assess whether the exposure levels achieved after once daily dosing in a hypothetical population of 180 HIV-infected children is comparable to historical values in children and adults. Here it is also assumed that similar efficacy can be inferred based on evidence of comparable exposures between dosing regimens.

In contrast to the typical bridging studies in which pharmacokinetic data is used to simply generate evidence of comparable drug exposure (figure 1), changes in dosing regimen require an additional assumption, i.e., that the underlying pharmacokinetic-pharmacodynamic relationships are dose and concentration-independent. Unfortunately, little has been done in this area to demonstrate that such a requirement is biologically plausible in most diseases where pharmacokinetic processes do not represent the rate limiting step for the onset and maintenance of response. According to the ICH guidelines, in these circumstances studies aimed at the evaluation of the pharmacological effects would usually be expected and the dose recommendations in children may be defined based on the biomarker response if established biomarkers are available, which can then be correlated with efficacy. This means that the use of pharmacokinetic-pharmacodynamic bridging in paediatric trials implies that even when pharmacodynamics cannot be used as a direct proxy for efficacy, it should suffice to demonstrate that the changes in viral load are correlated with systemic exposure.

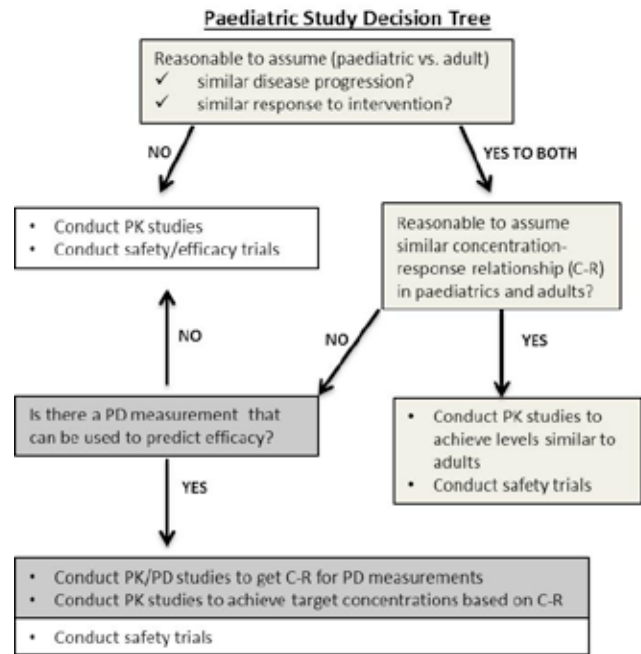


Figure 1 Paediatric study decision tree (adapted from FDA's Guidance to industry: exposure-response relationships - study design, data analysis, and regulatory applications; 1993). First, the disease and its time course or progression must be similar in adults and children. Second, the pharmacokinetic-pharmacodynamic relationships need to be comparable across the two populations. The third requirement refers to the use of efficacy endpoints in trial protocols which can be compared across population or for which a correlation has been demonstrated. If these three prerequisites are met, PK and safety studies are sufficient to infer efficacy. By contrast, evidence of efficacy and safety must be generated in the paediatric population when one or more requirements cannot be satisfied. PK: pharmacokinetic; PD: pharmacodynamic.

From a clinical perspective, our approach offers the opportunity for applying pharmacokinetic-pharmacodynamic relationships as the basis for the dose rationale. The availability of such data also offers the opportunity to explore hypothetical scenarios in large populations without the need to expose the patients to the actual protocol, thereby characterising in a quantitative manner the impact of drug-, disease- and patient-specific factors.

2.4. FORGIVENESS TO POOR ADHERENCE

In the previous sections of this thesis we have dealt with general issues regarding the predictive value of population models for bridging and extrapolation purposes and consequently for paediatric dose selection. We make clear that whilst the changes in pharmacokinetics due to

developmental growth may be easily characterised as long as covariate factors are included in a balanced manner, the use of pharmacokinetic-pharmacodynamic bridging based on inferences about comparable efficacy and safety imposes an additional assumption, i.e., that fluctuation in exposure levels are truly random in the population and that pharmacokinetic-pharmacodynamic relationships are time and concentration-independent. This assumption may be confounded by variable adherence patterns. Yet, one needs to keep in mind that perfect adherence to antiretroviral therapy is very difficult to achieve, especially in children (11). In fact, numerous studies have shown that non-adherence to antiretroviral therapy is one of the main causes of viral failure (12). In addition, it has been demonstrated that imperfect adherence can lead to sub-therapeutic drug levels, which may boost the development of drug resistance to one or several drugs in the treatment.

Here we propose a model-based approach to evaluate forgiveness of drug to treatment interruptions and deviations from the prescribed regimen, which cannot be assessed in a randomised controlled experimental protocol due to obvious ethical and clinical reasons. From a methodological standpoint, we show for the first time how clinical trial simulations can be used as a framework to evaluate complex adherence patterns and explore in a strictly quantitative manner its implications for efficacy and safety. Different mathematical and statistical models are combined together to describe the interaction between drug properties, disease characteristics and patient behaviour. Most importantly, we envisage the use of such a framework for virtual, rather than real populations.

The main objective of this section is therefore to assess adherence as a covariate effect on drug exposure using a range of scenarios. The forgiveness of a drug is the ability to achieve and maintain viral suppression despite sub-optimal adherence to the prescribed dosing regimen. This may depend on many factors, such as drug, viral and host properties. In Chapter 8, we investigate which properties of an antiretroviral drug might be related with its degree of forgiveness using a putative population of HIV-infected children (n=100). Three paradigm drugs belonging to different antiretroviral classes currently approved in children will be investigated, including a variety of patterns of non-adherence, which corresponds to the most common deviation(s) observed in protocol execution. Despite the somewhat complex framework, which involves pharmacokinetic, pharmacodynamic and disease models, the impact of poor adherence will be limited to the evaluation of the effects on viral load after monotherapy. In Chapter 9, the concept will be subsequently expanded to allow characterisation of the effects of poor adherence to a NNRTI-based regimen (efavirenz, lamivudine, abacavir). The probability to experience viral failure will be assessed in each scenario of non-adherence and a correlation between adherence and probability for the virus to mutate and become drug resistant will be included in the clinical trial simulation framework. In this chapter, we will also explore the feasibility to use pharmacokinetic-pharmacodynamic relationships as “proxy” for efficacy in the investigation of forgiveness of non-adherence. It is envisaged that evidence of comparable pharmacokinetic-

ic-pharmacodynamic relationships could be treated in a similar manner to pharmacokinetics in bioequivalence studies, i.e., a “proxy” for efficacy and safety.

The predictive value of pharmacokinetic-pharmacodynamic relationships as a proxy for efficacy will be evaluated for a range of adherence patterns. A clinical trial simulation will be performed to simulate treatment outcome in a virtual population (n=30) of children. Of interest is the relevance of changes in dosing regimen to pharmacokinetics and viral load. Based on this concept, in **Chapter 10** the forgiveness of non-adherence to a simplified dosing regimen (efavirenz, lamivudine and abacavir, all administered once daily) is compared with the forgiveness of non-adherence of the currently approved dosing regimen.

2.5. CONCLUSIONS AND PERSPECTIVES

A summary of the results and conclusions drawn from the various chapters is provided in **Chapter 11**. Here we show that three main topics regarding the development of antiretroviral paediatric therapy are intertwined, namely the dose, the dosing regimen and patient adherence to treatment. As such, they all contribute to success or failure of treatment. Despite the challenges in characterising such a complex interaction, throughout the various chapters, we highlight the relevance of evidence synthesis in paediatric drug development, as opposed to evidence generation as the basis for treatment optimisation in children. Pharmacokinetic-pharmacodynamic relationships can be used in conjunction with modelling and simulation to make inferences about efficacy and safety, overcoming many if not most of the ethical and technical constraints associated with clinical studies in children.

We also attempt to provide practical recommendations regarding the evaluation of models for the purposes of bridging and extrapolation of pharmacokinetic and pharmacodynamic data across populations. As highlighted in the first section of the thesis the challenge is to identify the factors that accurately describe the changes associated with developmental growth. Once the predictive performance of a model has been evaluated, its application in subsequent simulation scenarios must be considered carefully. The availability of a framework for clinical trial simulations offers not only the possibility to investigate the benefits and risks of a simplified dosing regimen, it also provides the basis for evaluating the role of patient behaviour. In this context we highlight that the importance of our investigation mainly relies on the use of an *in silico* approach to evaluate critical scenarios which could not be investigated in real-life due to obvious ethical issues. The possibility to derive quantitative measures of forgiveness may become critical for the development of novel antiretroviral compounds. Special attention is also given to the novelty of an *in silico* methodology for the exploration of non-adherence to therapy and to its potential applications to other chronic diseases.

References

1. Kenny, J., Mulenga, V., Hoskins, S., Scholten, F. & Gibb, D.M. The needs for HIV treatment and care of children, adolescents, pregnant women and older people in low-income and middle-income countries. *AIDS (London, England)* **26 Suppl 2**, S105-16 (2012).
2. King, J.R., Kimberlin, D.W., Aldrovandi, G.M. & Acosta, E.P. Antiretroviral pharmacokinetics in the paediatric population: a review. *Clinical pharmacokinetics* **41**, 1115-33 (2002).
3. Haberer, J. & Mellins, C. Pediatric adherence to HIV antiretroviral therapy. *Current HIV/AIDS reports* **6**, 194-200 (2009).
4. Mahmood, I. Prediction of drug clearance in children: impact of allometric exponents, body weight, and age. *Therapeutic drug monitoring* **29**, 271-8 (2007).
5. Ribbing, J. & Jonsson, E.N. Power, selection bias and predictive performance of the Population Pharmacokinetic Covariate Model. *Journal of pharmacokinetics and pharmacodynamics* **31**, 109-34 (2004).
6. Brendel, K. *et al.* Are population pharmacokinetic and/or pharmacodynamic models adequately evaluated? A survey of the literature from 2002 to 2004. *Clinical pharmacokinetics* **46**, 221-34 (2007).
7. Laventhal, N., Tarini, B.A. & Lantos, J. Ethical issues in neonatal and pediatric clinical trials. *Pediatric clinics of North America* **59**, 1205-20 (2012).
8. Cella, M., Zhao, W., Jacqz-Aigrain, E., Burger, D., Danhof, M. & Della Pasqua, O. Paediatric drug development: are population models predictive of pharmacokinetics across paediatric populations? *British journal of clinical pharmacology* **72**, 454-64 (2011).
9. Moyle, G. Once-daily therapy: less is more. *International journal of STD & AIDS* **14 Suppl 1**, 1-5 (2003).
10. Scherpbier, H.J., Bekker, V., Pajkrt, D., Jurriaans, S., Lange, J.M.A. & Kuijpers, T.W. Once-daily highly active antiretroviral therapy for HIV-infected children: safety and efficacy of an efavirenz-containing regimen. *Pediatrics* **119**, e705-15 (2007).

11. Simoni, J.M., Montgomery, A., Martin, E., New, M., Demas, P.A. & Rana, S. Adherence to antiretroviral therapy for pediatric HIV infection: a qualitative systematic review with recommendations for research and clinical management. *Pediatrics* **119**, e1371-83 (2007).
12. Gross, R., Bilker, W.B., Friedman, H.M. & Strom, B.L. Effect of adherence to newly initiated antiretroviral therapy on plasma viral load. *AIDS (London, England)* **15**, 2109-17 (2001).

SECTION II

Sample size, covariate distribution
and predictive performance
of pharmacokinetic models

COVARIATE EFFECTS AND POPULATION PHARMACOKINETICS OF LAMIVUDINE IN HIV-INFECTED CHILDREN

Chiara Piana, Wei. Zhao, Kim Adkison, David Burger, Evelyne Jacqz-Aigrain, Meindert Danhof and Oscar Della Pasqua

In press - Br J Clin Pharmacol

SUMMARY

Aim: Lamivudine is widely used as first-line therapy in HIV-infected children. Yet, the influence of developmental growth on drug exposure has not been fully characterised. Here we show how a comprehensive population pharmacokinetic model can be developed to account for the influence of demographic covariates on lamivudine exposure (i.e., AUC, C_{max}).

Methods: Data from 3 trials including children between 3 months and 13 years old were used in conjunction with a stepwise covariate selection to describe the pharmacokinetics across the overall population. Modelling was performed using nonlinear mixed-effects as implemented in NONMEM v.6.2. A stepwise forward inclusion and backward elimination procedure was used for covariate model building.

Results: A one-compartment model with first-order elimination was found to best describe the pharmacokinetics of lamivudine in children. The effect of body weight on clearance and volume of distribution was characterised by an exponential function. The exponent for the effect of weight on CL and V was 0.705 and 0.635, respectively. The estimate of CL for a patient of 17.6 kg (median body weight) was 16.5 L/h (CI 15.2-17.7), while the estimate of volume of distribution for a patient of 17.6 kg was 46.0 L (CI 42.4-49.5). There were no pharmacokinetic differences between the two formulations (tablet and solution). The predicted steady-state AUC₀₋₁₂ for twice daily dosing after a dose of 4 mg/kg ranged from 4.44 mg•h/L for children lighter than 14 kg to 7.25 mg•h/L for children heavier than 30 kg.

Conclusions: The use of a meta-analysis is critical to identify the correct covariate-parameter relationships, which must be assessed before a model can be applied for predictive purposes (e.g., defining dosing recommendations for children). In contrast to prior modelling efforts, we show that the covariate distribution in the target paediatric population must be considered.

3.1. INTRODUCTION

Lamivudine (3TC) is a nucleoside reverse transcriptase inhibitor (NRTI) widely administered as the nucleoside backbone in combination of highly active antiretroviral therapy to HIV-infected children. Lamivudine's mechanism of action is based on the competitive inhibition of the HIV reverse transcriptase. It is phosphorylated to an active metabolite that competes for incorporation into viral DNA. According to the latest WHO guidelines (1), lamivudine is administered as paediatric first-line therapy in combination with abacavir (ABC), with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). In fact, given its excellent record of efficacy, safety and tolerability in HIV-infected children, lamivudine is contained in practically all recommended combinations in paediatric antiretroviral therapy. In addition, it is a frequent component of fixed-dose, including low-cost, drug combinations.

Lamivudine is rapidly absorbed after oral administration and it is excreted primarily in the urine as unchanged drug (2–6). The intracellular triphosphate has a long half-life of 16 to 19 hours, as compared to the plasma lamivudine half-life of 5 to 7 hours (7). Lamivudine is currently administered to HIV-infected children based on body weight according to the dose of 8 mg/kg/day for children lighter than 14 kg, 150 mg/day from 14 to 21 kg, 225 mg/day from 21 to 30 kg, and 300 mg/day thereafter, all given twice a day.

Over the last years, various attempts have been made to describe the effect of developmental growth on the pharmacokinetics of lamivudine in children. Tremoulet *et al.* performed an extensive population pharmacokinetic analysis in infants between 3 days and 3 years (8); Burger *et al.* also investigated the influence of age on lamivudine pharmacokinetics in HIV-infected children, showing that in children of 6 years of age and younger, the recommended dose of 4 mg/kg twice daily leads to exposure levels lower than those observed in children ≥ 7 years of age and adults (9). These findings have prompted additional evaluation of the effects of developmental growth on the pharmacokinetics of lamivudine. In this context, focus has been given to the use of allometric models to characterise the effect of body weight on clearance. Bouazza *et al.* described the covariate effects in large group of children ($n=580$) aged between 2 days and 18 years (10), whilst Zhang *et al.* developed a population pharmacokinetic model in young children between 0.5 and 4.5 years (11). In all these studies, either small populations (i.e., group size) or narrow age ranges (i.e., population inclusion criteria) were used or the relationship between parameter and covariate was fixed *a priori*.

Bearing in mind the known issues associated with covariate selection when dealing with small datasets, we propose the use of a model-based meta-analysis for the analysis of the pharmacokinetics of lamivudine. Here we analyse data from three groups of HIV-infected children, focusing on the requirements for 1) accurately assessing the correlation between demographic covariates and pharmacokinetic parameters and 2) balance in the covariate distribution across the groups, without relying on *a priori* assumptions about the parameter-demographic covariate

correlation.

Given the need for a scientifically driven dose rationale in paediatric diseases (12), it can be anticipated that the correct identification of influential covariates on drug disposition is essential when a population pharmacokinetic model is used for simulations and dosing recommendation purposes (13–15).

Dosing recommendations should be therefore obtained without introducing bias due to factors such as unbalanced distribution of the covariates, or due to the small sample size available for data analysis. Such a bias may result into suboptimal dosing across different groups in the population and consequently lead to increased risk of toxicity or reduced efficacy. A deep understanding of the correlation between the demographic covariates and pharmacokinetic parameters is still required to assess the implications of developmental growth on drug exposure and, as a consequence, on the efficacy of lamivudine.

3.2. METHODS

Patients and samples

This investigation was a retrospective pooled analysis of data obtained from three studies: PENTA (Paediatric European Network for the Treatment of AIDS) 13; PENTA 15 and ARROW (AntiRetroviral Research fOr Watoto). The primary objectives of these studies were to compare the pharmacokinetics of once daily versus twice daily lamivudine regimens in HIV type-1-infected children. PENTA 13 and PENTA 15 were conducted in European children aged from 2-13 years and from 3 months-3 years, respectively. The ARROW study was conducted in Uganda with children aged 3-12 years. The studies have been conducted in full conformance with the principles of the Declaration of Helsinki and with the local laws and regulations concerning clinical trials. The protocol and the informed consent documents for each study have been formally approved by the relevant research ethics committee of each clinical site and by a national ethics body. In total data from 77 paediatric patients were available (19 from PENTA 13 study (16), 18 from PENTA 15 study (17) and 40 from the ARROW trial (18)). The analysis population consisted of male and female patients across the age range between 3 months and 13 years (median age 5.79 years), and weight between 7.43 and 61.3 kg (median weight 17.6 kg). Demographic details are summarised in Table 1. In total 1184 blood samples were available for pharmacokinetic modelling, with 9 samples below the quantification limit.

Table 1 Summary of demographic characteristics

	Penta13	Penta 15	ARROW	Integrated analysis
SUBJECTS	19	18	40	77
STEADY STATE	Yes	Yes	Yes	
MEDIAN AGE (years)	5.79	1.91	7.56	5.79
MIN (years)	2.14	0.42	3.5	0.42
MAX (years)	12.84	2.81	12.57	12.84
MEDIAN WEIGHT (kg)	21.75	11.71	20.125	17.6
MIN (kg)	12.5	7.43	14	7.43
MAX (kg)	61.3	16.1	30	61.3
CREATININE CLEARANCE (mL/min)	81.72	59.9	63.89	95.59
MIN (mL/min)	41.25	31.99	50.43	31.99
MAX (mL/min)	199.59	87.58	168.32	199.59
ETHNICITY	17 black, 2 others	14 black, 4 others	40 black	71 black, 6 others

Assay of lamivudine

For the PENTA13 and PENTA15 studies, plasma concentrations of lamivudine were determined by high performance liquid chromatography assay with UV detection (HPLC-UV) with a lower limit of quantification (LLOQ) of 0.015 mg/L (19). For the ARROW study, the high performance liquid chromatography assay with tandem mass spectrometry detection (HPLC-MS/MS) method was used, which had a LLOQ of 0.0025 mg/L.

Population pharmacokinetic analysis

The pharmacokinetic analysis was done in two steps:

1. Development of the population pharmacokinetic model using a subset of two studies (PENTA 13 and PENTA 15 studies) to allow for an initial assessment of model stability and predictive performance.
2. Integrated pharmacokinetic analysis of the patient data from all three studies, followed by model validation, as implemented by standard graphical and statistical methods.

Model Building

Nonlinear mixed effects modelling was performed in NONMEM version 6.2 (Icon Development Solutions, USA)(20). Model building criteria included: (i) successful minimisation, (ii) standard error of estimates, (iii) number of significant digits, (iv) termination of the covariance step, (v) correlation between model parameters and (vi) acceptable gradients at the last iteration (21).

Fixed and random effects were introduced into the model in a stepwise manner. A parameter value of an individual i (post hoc value) is therefore given by the following equation:

$$\theta_i = \theta_{TV} * e^{\eta_i}$$

in which θ_{TV} is the typical value of the parameter in the population and η_i is the variability between subjects which is assumed to follow a normal distribution with mean zero and variance ω^2 . Residual variability, which comprises measurement and model error, was described with a proportional error model. This means for the j^{th} observed concentration of the i^{th} individual the relation Y_{ij} :

$$Y_{ij} = F_{ij} + \epsilon_{ij} * W$$

Where F_{ij} is the predicted concentration and ϵ_{ij} the random variable with mean zero and variance σ^2 . W is a proportional weighing factor for ϵ .

Goodness of fit was assessed by graphical methods, including population and individual predicted vs. observed concentrations, conditional weighted residual vs. observed concentrations

and time, correlation matrix for fixed vs. random effects, correlation matrix between parameters and covariates and normalised predictive distribution error (NPDE) (22). Comparison of hierarchical models was based on the likelihood ratio test. A superior model was also expected to reduce inter-subject variability terms and/or residual error terms.

Covariate analysis

Continuous and categorical covariates were tested during the analysis. The relationship between individual pharmacokinetic parameters (*post-hoc* or conditional estimates) and covariates was explored by graphical methods (plot of each covariate vs. each individual parameter). Relevant demographic covariates (body weight, age, height, creatinine clearance) were entered one by one into the population model (univariate analysis). Given that different lamivudine formulations were administered in the trials, formulation was also treated as a covariate. After all significant covariates had been entered into the model (forward selection), each covariate was removed (backward elimination), one at a time. The model was run again and the objective function recorded. The likelihood ratio test was used to assess whether the difference in the objective function between the base model and the full (more complex) model was significant. The difference in -2Log likelihood (DOBJF) between the base and the full model is approximately χ^2 distributed, with degrees of freedom equal to the difference in number of parameters between the two hierarchical models. Because of the exploratory nature of this investigation, for univariate analyses, additional parameters leading to a decrease in the objective function of 3.84 was considered significant ($p < 0.05$). During the final steps of the model building, only the covariates which resulted in a difference of objective function of at least 7.88 ($p < 0.005$) were kept in the final model.

Model validation

The validation of the final model was based on graphical and statistical methods. Given the importance of the validation procedures for the subsequent use of a model for simulation purposes, in this study we used different tools to validate the model. First, a bootstrap procedure was performed in PsN v2.30 (University of Uppsala, Sweden) (23). Bootstrap was used to identify bias, stability and accuracy of the parameter estimates (standard errors and confidence intervals). PsN does so by generating a set of new datasets by sampling individuals with replacement from the original dataset, and fitting the model to each new dataset.

Subsequently, parameter estimates were used to simulate plasma concentrations in paediatric HIV patients with similar demographic characteristics, dosing regimens and sampling scheme as in the original clinical studies. Mirror plots were then applied to evaluate the degree of similarity between the original fit and the pattern obtained from the fitting of the simulated data sets using the final pharmacokinetic model.

In addition to the graphical analysis, posterior predictive check was performed using AUC (area under the plasma concentration vs. time curve) and C_{max} (peak plasma concentration) as a measure of model performance. AUC and C_{max} values were calculated non-compartmentally by trapezoidal method from simulations of 1000 data sets with the same demographic characteristics, dosing regimens and sampling scheme as in the original clinical studies. The distribution of model-predicted AUC and C_{max} values were presented for geometric mean, lower and upper boundaries of the 95% confidence intervals and compared to the findings from non-compartmental analysis in the two clinical studies. Model performance was demonstrated by the location of the original estimates across the predicted distribution (histograms).

3.3. RESULTS

Population pharmacokinetic modelling

The results shown in this paper are derived from the analysis of the combined datasets from three studies. A one-compartment pharmacokinetic disposition model with first order absorption was fitted to the plasma concentration vs. time data derived from the three populations. Inter-individual variability was identified for CL, V and K_a. In all three studies used in our investigation the patients received lamivudine according to once and twice daily dosing regimen. Therefore inter-occasion variability on CL and K_a was included in the model to quantify potential differences in parameter estimates between the two dosing regimens. The residual error was described using a combined model including a weighing factor for the variance estimate, which showed a better fit of the data compared with a simple combined error model. CL and V were found to increase with body weight. An exponential function best described the correlation between these pharmacokinetic parameters and body weight. The exponent for the effect of weight on CL was 0.705 and the exponent for the effect of weight on V was 0.635.

It should be pointed out that both body weight and age showed an influence on lamivudine clearance and volume of distribution. However, based on the magnitude of the changes in objective function (i.e., statistical criteria used for model building), body weight was found to be more influential than age on lamivudine pharmacokinetics. In addition to the statistical criteria, graphical diagnostics were used to assess the goodness-of-fit. As shown in figures 1, population and individual predictions are unbiased.

Although concentrations below the quantification limit were present at time 0 and 24h, the predicted mean concentrations did not significantly differ from the observed mean concentrations (0.081 mg/L vs. 0.098 mg/L at time 0 h and 0.059 mg/L vs. 0.061 mg/L at 24 hours after dose).

Model validation

The validation procedure has been performed for twice daily and once daily data separately to ensure accurate characterisation of the data irrespective of the dosing regimen. The visual predictive check (VPC) (figure 1) indicated model stability and absence of significant bias in the estimates for fixed and random effects. Bootstrapping was also performed as part of the validation procedures. All runs carried out (n= 500) were successful. As shown in Table 2, the final parameter estimates and their confidence intervals were very similar to original fitting. Given that few patients between 3 to 24 months of age were included in the analysis (n=11), scatter plots of observed vs. model predicted AUC and Cmax for these subjects are shown to illustrate model performance in young children (figure 2). The predictive performance of the model in subsequent simulations was deemed critical to achieve the objective of our analysis. To this purpose, mirror plots were used to assess whether the variance and covariance structures have been well characterised. Mirror plots explore whether model parameters can accurately replicate the findings in the original study, enabling therefore further assessment of the covariate effects on dosing regimen and dose recommendations.

To complete the validation, a graphical summary of model performance across different weight ranges was used to assess the predicted distribution for the variable of interest [AUC₀₋₁]. As shown in figure 3, the predicted AUC distribution encompasses the exposure observed in the original dataset.

Table 2 Summary of pharmacokinetic parameter estimates from the final model. Parameters are presented only for the final model and not for the initial model built using data from PENTA 13 and PENTA 15 studies.

Parameter ¹	Population Estimate	%CV	Bootstrap Mean (95%CI)
Clearance (CL) CL/F= $\theta_1 \cdot (BW/med)^{\theta_5}$ (intercept) L/h (exponent) L/h/kg	16.5 0.705	3.8 14.9	16.5 (15.2-17.7) 0.701(0.498-0.911)
Volume (V) V/F= $\theta_2 \cdot (BW/med)^{\theta_6}$ (intercept) L (exponent) L/kg	46.0 0.635	3.7 14.0	46.0(42.4-49.5) 0.625(0.461-0.809)
Absorption rate constant (Ka) 1/h	3.68	15.9	3.86 (1.92-5.43)
ALAG1 h	0.755	4.5	0.755 (0.658-0.851)

Weighing factor in \$ERR	-2.69	14.7	-2.73 (-4.13,-1.24)
Interindividual variability			
$\eta_{CL/F}$ variance	26% ²	30.3	25.4 (17.8-32.2)
$\eta_{V/F}$ variance	19% ²	52.8	18.7(9.48-24.49)
η_{Ka} variance	66% ²	37.8	66.3 (31.3 -88.1)
Inter-occasion variability			
OCCCL	27% ³	27.2	26.8 (15.5 -34.8)
OCCKa	72% ³	42.5	72.1 (41.6-94.1)
OCCV	20% ³	32.73	19.7 (10.5 -26.3)
Residual Error			
Additive error mg/l	7%	21.48	7.07 (4.5-8.4)

1. Population parameter point-estimates for the full one-compartment model and 95%CI and %CV from a non-parametric bootstrap are presented.
2. Value in parentheses represents the interindividual variability of the pharmacokinetic parameters calculated as the square root of $\Omega \times 100\%$.
3. Value in parentheses represents the inter-occasion variability of the pharmacokinetic parameters calculated as the square root of the $\Omega \times 100\%$

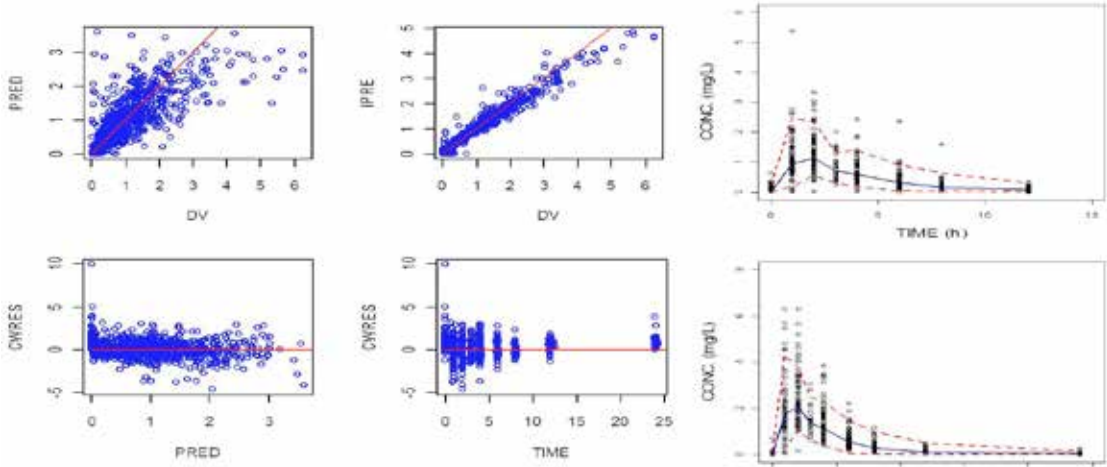


Figure 1 (a) Goodness-of-fit (Left). Left upper panel shows the population prediction (PRED) vs. observed concentration values (DV). Right upper panel shows individual predictions (IPRED) vs. observed concentration values (DV). Left lower panel shows conditional weighted residuals (CWRES) vs. population predictions (PRED). Right lower panel shows conditional weighted residuals (CWRES) vs. time (TIME). Solid line represents the identity line. (b) Visual predictive check (VPC) of the population PK model for lamivudine (Right). The dots represent observed concentrations, the dotted lined represent the 5th and 95th percentiles of the simulated values. The solid blue line represents the median of the simulated profiles. The VPC is presented for the data following twice daily (upper panel) and once daily (lower panel) dosing.

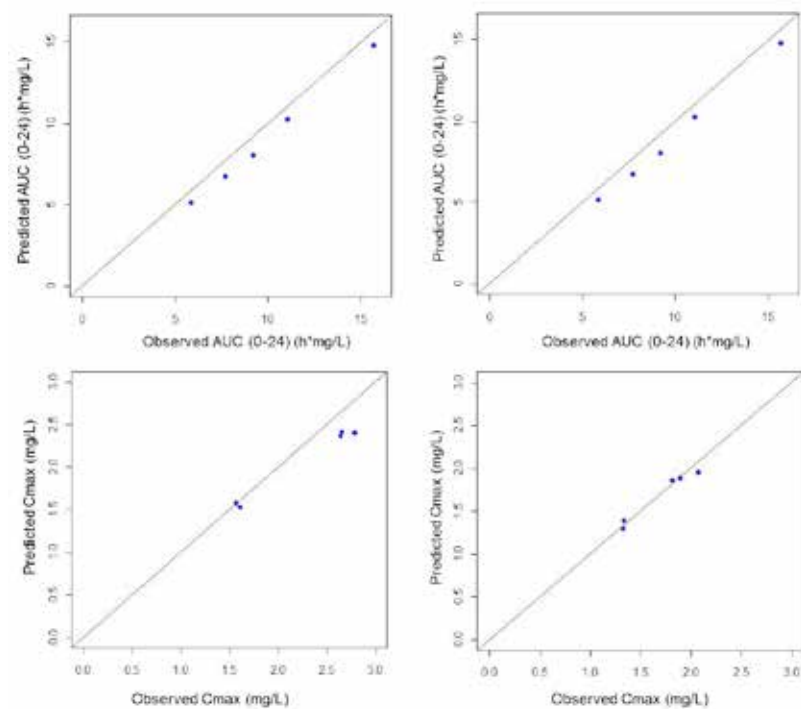


Figure 2 Scatter plots of observed vs. model predicted AUC_{0-24} (top panels) and C_{max} (bottom panels) for children younger than 24 months. Left panels show children from 0 to 12 months, whereas children from 12 to 24 months are shown on the right panels.

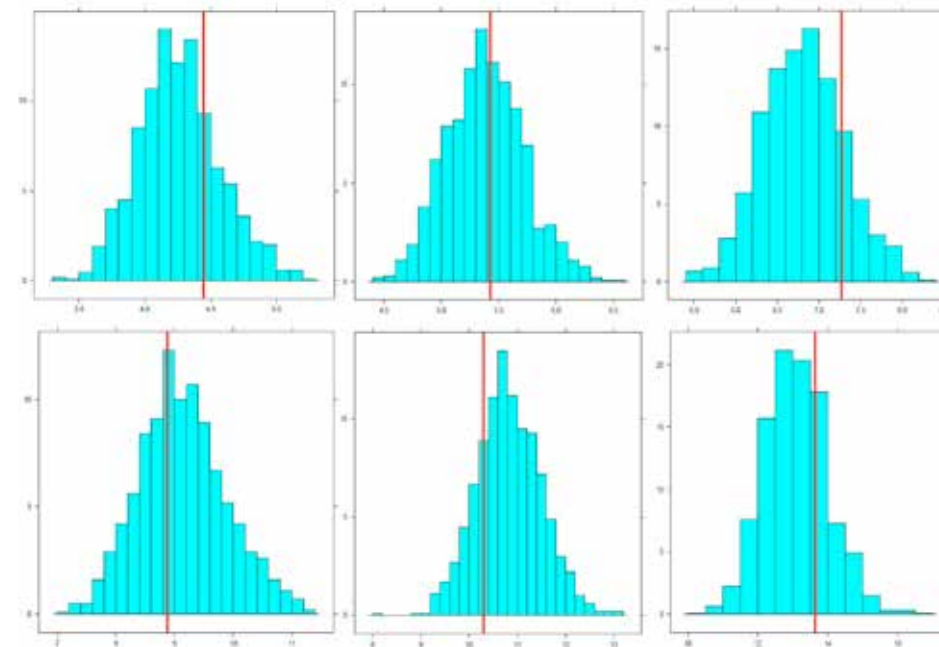


Figure 3 Distribution of the model-predicted area under the plasma concentration vs. time curve [$AUC_{0-\infty}$] (1000 replicate trials) compared to the original dataset. The left panels show $AUC_{0-\infty}$ predictions for children weighing less than 14 kg, middle panels show $AUC_{0-\infty}$ predictions for children from 14 to 21 kg and right panels show $AUC_{0-\infty}$ predictions for children weighing more than 21 kg. These weight boundaries were defined according to dosing recommendations available in the approved label. Predictions for twice and once daily doses are shown in the upper and lower panels, respectively. The solid line represents the geometric mean of the observed $AUC_{0-\infty}$ in the three sub-groups for each dosing regimen. $AUC_{0-\infty} = AUC_{0-12}$ for twice daily dosing and $AUC_{0-\infty} = AUC_{0-24}$ for once daily.

3.4. DISCUSSIONS AND CONCLUSIONS

Pharmacokinetic Model for the Paediatric Population

A model-based approach has been applied in our study to describe the pharmacokinetics of lamivudine in HIV-infected children across a wide age range. A one-compartment model with first order absorption was found to best describe lamivudine pharmacokinetics, which is consistent with previous studies in adults and children (5, 8). In our analysis body weight was the only covariate found to influence lamivudine apparent clearance and volume of distribution, which is also in agreement with earlier investigations (5, 8, 10). However, differently from the study in adults, creatinine clearance was not found to have an effect on lamivudine apparent clearance, probably because its effect was confounded by body weight. Apparent clearance estimates in our study were very similar to literature findings in children (16.5 vs. 16.9 L/h). In addition, it was not possible to find a significant effect of the formulation on relevant pharmacokinetic parameters or to estimate a relative bioavailability of the two formulations. Similar results were reported previously by Bouazza (24), whereas Kasirye *et al.* (25) showed in a study with 19 children (aged 1.8 to 4 years) that lamivudine exposure was 55% higher after administration of the solid dosage form (i.e., tablet) as compared to the liquid formulation. Such differences may be partly due to dose approximation in scored tablet as compared to the precise dose administration of the solution.

Identification of covariates in lamivudine pharmacokinetics in children

Our meta-analysis using three groups of HIV-infected children (see Table 1) included model building and validation steps to ensure predictive performance in subsequent application of the model, as e.g., in clinical trial simulations.

In contrast to common practice, an integrated analysis of the full population was performed after preliminary model-building based on a subset of the full population. Such a method was chosen to assess model stability and confirm the selection and magnitude of the effect of influential covariates. This approach can be particularly useful in paediatric studies, given the difficulties in identifying the correct demographic covariates, which are often highly correlated with each other (13, 14, 26). Given that a wrong decision in covariate selection may affect future dosing recommendation, special attention should be paid to covariate model building. As shown in a previous study, the stepwise approach commonly used for covariate selection may introduce selection and omission bias in the model when the dataset used during the analysis is small (27). In fact, in small datasets the distribution of the covariates may not allow identification of a correlation between the covariate and the pharmacokinetic parameters.

In our analyses, the initial lamivudine model accurately predicted the pharmacokinetic profiles of the group which was not used for initial model building (results not shown). The same parameter-covariate correlations were identified when the model was re-evaluated using the

full paediatric population. It is important to point out that the correlation between clearance and body weight is exponential. For example, the apparent clearance has a median value of 9.33 L/h for a child weighing 10 kg. It increases to 16.55 L/h in children whose weight is 20 kg, but only increases by an additional 1.27 L/h in children of 30 kg (17.82 L/h).

A separate analysis of the data from the ARROW trial (age range 3 to 12 years) was also performed and a one-compartment model with first order absorption and elimination was identified to best describe this subset of data. Very interestingly, none of the demographic covariates available was found to be significantly correlated to the pharmacokinetic parameters. Furthermore, diagnostic measures, such as the visual predictive check of the model, were not able to show any inaccuracy or bias in model-based predictions of the data (figure 5). These results suggest that the model could be used subsequently for dosing recommendation purposes. However, its use would yield incorrect model-based predictions in a different population since the correct parameter-covariate relationship was not identified during covariate model building. This finding strongly underlines the importance of an integrated data analysis and the risk of inaccurate covariate selection when only a part of the full population is available for analysis.

Limitations of current approaches in paediatric dosing

Many examples are available in literature of population pharmacokinetic analyses based on less than 40 patients (28–31). In such small populations an unbalanced covariate distribution may lead to the identification and selection of wrong covariate-parameter relationships and, in turn, to wrong model-based predictions when applying the model to a different population (i.e., extrapolation). Many experts in paediatric pharmacology claim to be able to define the type and magnitude of the effect of a covariate on pharmacokinetic parameters; however they do not take into account that the parameter-covariate correlation may be biased by the covariate distribution in that particular group of children and as such should not be used in a different population. In these circumstances, one should talk about a data-driven approach, in the sense that the model is able to correctly describe the data (as shown in our analysis by the visual predictive check in figure 4), but is not able to predict correctly the variable of interest in a different population. It is also worth mentioning that such a hidden bias is not addressed by simply increasing the sample size as often is the case in pooled analysis of patients undergoing therapeutic drug monitoring. Meta-analyses should therefore be the preferred method in paediatric pharmacokinetics to avoid model misspecification and consequently expose children to suboptimal drug levels or to a higher risk of toxicity. When sufficient paediatric data are not available, one should consider overcoming the limitations of small populations by incorporating prior information from pharmacokinetic parameters in adults and include them in the model, as suggested by Cella *et al* (12). There are also other research groups, who choose not to use pharmacokinetic modelling for the analysis for drug exposure and dose selection in children. Instead, they prefer to solely rely on non-compartmental analysis, ignoring the issues highlighted above. The use

of a model-based approach presents significant advantages compared to non-compartmental analysis, which cannot be overlooked from a scientific and ethical point of view.

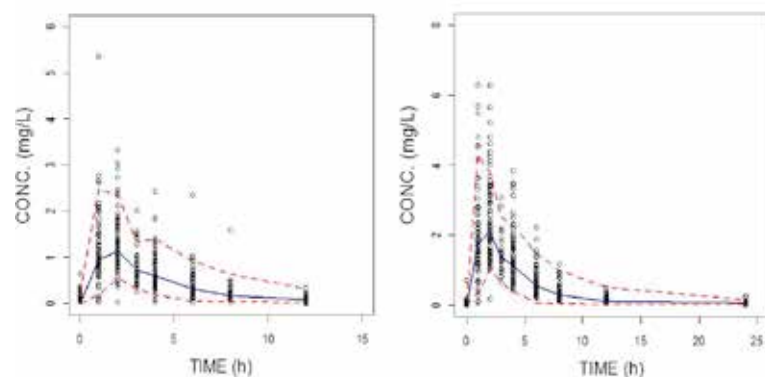


Figure 4 Visual predictive check (VPC) of the population model for lamivudine using only the data from the ARROW trial. The dots represent observed concentrations, the dotted lines represent the 5th and 95th percentiles of the simulated values. The solid line represents the median of the simulated profiles. The VPC is presented for the data following twice daily (left) and once daily (right) dosing.

Clinical implications of an integrated population analysis for accurate dosing recommendation

Given that drug exposure drives efficacy, it should be clear that model misspecification may lead to incorrect dosing recommendations. The identification of the correct covariate-parameter relationships is therefore crucial to accurately predict drug exposure across different groups in the paediatric population. Yet, this issue is further compounded by current prescription practices.

The role of covariate-parameter correlations is apparently even more important when exploring changes in dosing regimen. For instance, we could not investigate the effect of obesity in this population. However, given the low lipophilicity of lamivudine (which is water soluble), we anticipate no major impact of obesity on its pharmacokinetics. It is conceivable that doses based on lean body mass might be required for very obese patients, as drug distribution and metabolism would not increase proportionally to total body weight.

How to dose a drug in children remains a very debatable subject. Whereas normalisation of the dose by body weight makes prescription easy and reduces the risk for prescription errors, deriving dose recommendations without a thorough understanding of drug disposition in children has been proven to be unsafe and harmful (32). Clearly, the effect of developmental growth on pharmacokinetics is a nonlinear phenomenon and as such can be best described by a model-based approach. However, modelling and simulation techniques should be used with caution. Too little attention has been paid so far to the implications of unbalanced covariate distributions on pharmacokinetic analyses outcome, as shown by the elevated number of examples available in literature. We are fully aware of the challenges in performing paediatric trials and in collecting clinical data in children. These difficulties must not prompt us to neglect the problems caused by small datasets, which may lead to the wrong dose selection. The use of meta-analyses, i.e., combined datasets from available clinical trials in children is strongly encouraged to avoid erroneous predictions of the paediatric dose.

Limitations in our approach

It is important to mention that lamivudine plasma concentrations represent a limited marker of drug exposure, as it is the intracellular lamivudine triphosphate metabolite that becomes pharmacologically active. Unfortunately adequate sampling for determination of intracellular concentrations of nucleoside transcriptase inhibitor triphosphate is logistically and technically difficult (33). Furthermore the volume of blood needed to measure intracellular lamivudine triphosphate concentrations with current technology makes serial evaluations impractical for paediatric patients.

Conclusions

The clinical relevance of a pharmacokinetic model depends on the generalisability of the covariate model across the overall population. Here we have shown that covariate effects may be under or overestimated if the available data do not support accurate identification of the correlation between pharmacokinetic parameter and covariate. Unbalanced distribution of covariates may result in hidden bias and yield inaccurate dosing recommendations in children. In addition, our work shows that the concept of pharmacokinetic bridging has been met for lamivudine, in that the dosing corrected by body weight does account for developmental growth, yielding comparable systemic exposure throughout the population older than 3 months of age.

References

1. Antiretroviral therapy for HIV infection in infants and children. Available at <http://www.who.int/hiv/pub/paediatic/paed-prelim-summary.pdf> (last accessed 4 May 2013)
2. Johnson, M.A., Moore, K.H., Yuen, G.J., Bye, A. & Pakes, G.E. Clinical pharmacokinetics of lamivudine. *Clinical pharmacokinetics* **36**, 41-66 (1999).
3. Kumar, P.N. & Patel, P. Lamivudine for the treatment of HIV. *Expert opinion on drug metabolism & toxicology* **6**, 105-14 (2010).
4. Lewis, L.L. *et al.* Lamivudine in children with human immunodeficiency virus infection: a phase I/II study. The National Cancer Institute Pediatric Branch-Human Immunodeficiency Virus Working Group. *The Journal of infectious diseases* **174**, 16-25 (1996).
5. Moore, K.H. *et al.* Pharmacokinetics of lamivudine administered alone and with trimethoprim-sulfamethoxazole. *Clinical pharmacology and therapeutics* **59**, 550-8 (1996).
6. Yuen, G.J., Morris, D.M., Mydlow, P.K., Haidar, S., Hall, S.T. & Hussey, E.K. Pharmacokinetics, absolute bioavailability, and absorption characteristics of lamivudine. *Journal of clinical pharmacology* **35**, 1174-80 (1995).
7. Moore, K.H. *et al.* The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. *AIDS (London, England)* **13**, 2239-50 (1999).
8. Tremoulet, A.H. *et al.* Population pharmacokinetics of lamivudine in human immunodeficiency virus-exposed and -infected infants. *Antimicrobial agents and chemotherapy* **51**, 4297-302 (2007).
9. Burger, D.M. *et al.* Age-dependent pharmacokinetics of lamivudine in HIV-infected children. *Clinical pharmacology and therapeutics* **81**, 517-20 (2007).
10. Bouazza, N. *et al.* Developmental pharmacokinetics of lamivudine in 580 pediatric patients ranging from neonates to adolescents. *Antimicrobial agents and chemotherapy* **55**, 3498-504 (2011).
11. Zhang, C. *et al.* Population pharmacokinetic model for adherence evaluation using lamivudine concentration monitoring. *Therapeutic drug monitoring* **34**, 481-4 (2012).

12. Cella, M., Gorter de Vries, F., Burger, D., Danhof, M. & Della Pasqua, O. A model-based approach to dose selection in early pediatric development. *Clinical pharmacology and therapeutics* **87**, 294-302 (2010).
13. Mandema, J.W., Verotta, D. & Sheiner, L.B. Building population pharmacokinetic--pharmacodynamic models. I. Models for covariate effects. *Journal of pharmacokinetics and biopharmaceutics* **20**, 511-28 (1992).
14. Anderson, B.J., Allegaert, K. & Holford, N.H.G. Population clinical pharmacology of children: modelling covariate effects. *European journal of pediatrics* **165**, 819-29 (2006).
15. Anderson, B.J., Allegaert, K. & Holford, N.H.G. Population clinical pharmacology of children: general principles. *European journal of pediatrics* **165**, 741-6 (2006).
16. Bergshoeff, A. *et al.* Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
17. Paediatric European Network for Treatment of AIDS (PENTA). Pharmacokinetic study of once-daily versus twice-daily abacavir and lamivudine in HIV type-1-infected children aged 3-<36 months. *Antiviral therapy* **15**, 297-305 (2010).
18. Musiime, V. *et al.* Pharmacokinetics and acceptability of once- versus twice-daily lamivudine and abacavir in HIV type-1-infected Ugandan children in the ARROW Trial. *Antiviral therapy* **15**, 1115-24 (2010).
19. Verweij-van Wissen, C.P., Aarnoutse, R.E. & Burger, D.M. Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **816**, 121-9 (2005).
20. Beal SL. NONMEM user's guide. NONMEM Project Group, University of California, San Francisco. (2011).
21. Duffull, S.B., Kirkpatrick, C.M.J., Green, B. & Holford, N.H.G. Analysis of population pharmacokinetic data using NONMEM and WinBUGS. *Journal of biopharmaceutical statistics* **15**, 53-73 (2005).
22. Brendel, K., Comets, E., Laffont, C., Laveille, C. & Mentr , F. Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. *Pharmaceutical research* **23**, 2036-49 (2006).
23. Lindbom, L., Ribbing, J. & Jonsson, E.N. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. *Computer methods and programs in biomedicine* **75**, 85-94 (2004).
24. Bouazza, N. *et al.* Is the recommended once-daily dose of lamivudine optimal in West African HIV-infected children? *Antimicrobial agents and chemotherapy* **54**, 3280-6 (2010).
25. Kasirye, P. *et al.* Pharmacokinetics of antiretroviral drug varies with formulation in the target population of children with HIV-1. *Clinical pharmacology and therapeutics* **91**, 272-80 (2012).
26. Mager, H. Covariate selection in population pharmacokinetics/pharmacodynamics. *International journal of clinical pharmacology and therapeutics* **42**, 187-8 (2004).
27. Ribbing, J. & Jonsson, E.N. Power, selection bias and predictive performance of the Population Pharmacokinetic Covariate Model. *Journal of pharmacokinetics and pharmacodynamics* **31**, 109-34 (2004).
28. Zuppa, A.F., Nicolson, S.C., Barrett, J.S. & Gastonguay, M.R. Population pharmacokinetics of pentobarbital in neonates, infants, and children after open heart surgery. *The Journal of pediatrics* **159**, 414-419. e1-3 (2011).
29. Guilhaumou, R. *et al.* Population pharmacokinetics and pharmacogenetics of vincristine in paediatric patients treated for solid tumour diseases. *Cancer chemotherapy and pharmacology* **68**, 1191-8 (2011).
30. Bressolle, F., Khier, S., Rochette, A., Kinowski, J.M., Dadure, C. & Capdevila, X. Population pharmacokinetics of nalbuphine after surgery in children. *British journal of anaesthesia* **106**, 558-65 (2011).
31. Bouillon-Pichault, M. *et al.* Topiramate pharmacokinetics in infants and young children: contribution of population analysis. *Epilepsy research* **93**, 208-11 (2011).
32. Cella, M., Knibbe, C., Danhof, M. & Della Pasqua, O. What is the right dose for children? *British journal of clinical pharmacology* **70**, 597-603 (2010).
33. Fletcher, C.V. *et al.* Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. *AIDS (London, England)* **14**, 2137-44 (2000).

POPULATION PHARMACOKINETICS OF ABACAVIR IN INFANTS, TODDLERS AND CHILDREN

Chiara Piana, Wei Zhao, Meindert Danhof, David Burger,
Oscar Della Pasqua, Evelyne Jacqz-Aigrain

Br J Clin Pharmacol. 2013

SUMMARY

Aims: To characterise the pharmacokinetics of abacavir in infants, toddlers and children, and assess the influence of covariates on drug disposition across these populations.

Methods: Abacavir concentration data from three clinical studies in HIV-infected children (n=69) were used for model building. The children received either a weight-normalised dose of 16 mg/kg/day or the WHO recommended dose based on weight-bands. A population pharmacokinetic analysis was performed using NONMEM v6. The influence of age, gender, body weight and formulation was evaluated. The final model was selected according to graphical and statistical criteria.

Results: A two-compartmental model with first-order absorption and first-order elimination best described the pharmacokinetics of abacavir. Body weight was identified as significant covariate influencing the apparent oral clearance and volume of distribution. Predicted steady-state C_{max} and AUC₀₋₁₂ of standard twice daily regimen were 2.5 mg/L and 6.1 mg•h/L for toddlers and infants, and 3.6 mg/L and 8.7 mg•h/L for children, respectively. Model-based predictions showed that equivalent systemic exposure was achieved after once and twice daily dosing regimens. There were no pharmacokinetic differences between the two formulations (tablet and solution). The model demonstrated good predictive performance for dosing prediction in individual patients and as such can be used to support therapeutic drug monitoring in conjunction with sparse sampling.

Conclusions: The disposition of abacavir in children appears to be affected only by differences in size, irrespective of the age of the patient. Maturation processes of abacavir metabolism in younger infants should be evaluated in further studies to demonstrate the potential impact of ontogeny.

4.1. INTRODUCTION

Abacavir is a potent nucleoside reverse transcriptase inhibitor (NRTI), prescribed in combination with other antiretroviral agents (nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs)) for the treatment of human immunodeficiency virus (HIV) infection in both paediatric and adult patients (1,2). It is marketed for paediatric patients from 3 months to 16 years at the dose of 8 mg/kg twice daily, up to a maximum of 300 mg twice daily.

Abacavir is well absorbed following oral administration and distributed into body tissues, including the central nervous system. It is extensively metabolized by the liver and less than 2% is excreted as unchanged drug in the urine. The two major catabolic pathways include oxidation by alcohol dehydrogenase (ADH) and conjugation by uridine diphosphate glucuronyltransferase (UGT), resulting in inactive carboxylate and glucuronide metabolites (3,4). The antiviral activity of abacavir results from its intracellular activation to carbovir triphosphate (CBV-TP). CBV-TP competes with the endogenous nucleotide 2'-deoxyguanosine triphosphate (dGTP) for incorporation into the nucleic acid chain and terminates the DNA chain by preventing addition of new bases (5). The endpoint for efficacy, as indicated by the change from baseline in viral load (plasma HIV-1 RNA) and T cells count rise was significantly correlated with area under the concentration-time curve (AUC) (6). The AUC_{0-12} value of 6.02 mg•h/L was set as target exposure both in adults and children (7).

The pharmacokinetics of abacavir has been previously investigated in children (8-15). However these studies were based on either a small number of patients, sparse sampling or narrow age range of the children, which renders difficult the assessment of the role of developmental factors on drug disposition. Accurate characterisation of these factors may allow not only further assessment of the individual dosing requirements across different age groups, but also insight into processes determining maturation and metabolic capacity, which may be deemed drug-independent. In this investigation, we make use of a model-based approach to analyse three different studies in children across a wide age range, with the objective of obtaining more reliable prediction of pharmacokinetic profiles in individual patients. In addition, given the availability of tablet and solution dosage forms, this analysis offered us the opportunity to explore the potential influence of formulation on paediatric pharmacokinetic parameters.

4.2. METHODS

Clinical trials

The data were obtained from three studies: PENTA (Pediatric European Network for the Treatment of AIDS) 13, PENTA 15 and a pharmacokinetic sub-study within the main ARROW (AntiRetroviral Research fOr Watoto) trial (8-10). Briefly, the primary objectives of these studies were to compare the pharmacokinetics of once daily versus twice daily of abacavir and lamivudine in HIV type-1-infected children. The European studies PENTA 13 and PENTA 15 were conducted in children aged from 2-13 years and from 3 months-3 years, respectively. The ARROW pharmacokinetic sub-study was conducted in Uganda with children aged 3-12 years. In total, sixty-nine children were included in this population pharmacokinetic meta-analysis. The mean (SD) age was 5.74 (3.40) (range 0.42 – 12.84) years and the mean (SD) weight was 18.7 (8.0) (range 7.6 – 60.9) kg. Pharmacokinetic samples were obtained at steady-state at time T0 (immediately before administration) and T1, T2, T3, T4, T6, T8 and T12 h after administration for the twice daily regimen and an additional sample at T24 h for the once daily regimen. A summary of trial design, dosage regimens, and patient characteristics are presented in Table 1. The studies have been conducted in full conformance with the principles of the Declaration of Helsinki and with the local laws and regulations concerning clinical trials. The protocol and the informed consent documents for each study have been formally approved by the relevant research ethics committee of each clinical site and by a national ethics body.

Bioanalysis

For the PENTA13 and PENTA15 studies, plasma concentrations of abacavir were determined by high performance liquid chromatography assay with UV detection (HPLC-UV). The details of the analytical method have been reported (8,9). The lower limit of quantification (LLOQ) was 0.015 mg/L. Within-day and between-day variability were 1.1–1.9% and 0.16–2.3%, respectively. For ARROW study, plasma concentrations of abacavir were determined using validated HPLC/MS/MS method by GlaxoSmithKline (Research Triangle Park, NC, USA). The LLOQ was 0.0025 mg/L (10).

Pharmacokinetic modelling

Pharmacokinetic analysis was carried out using the nonlinear mixed effects modelling program NONMEM v6 (V2.0; Icon Development Solutions, USA) (16). First order conditional estimation (FOCE) method with interaction option was used to estimate pharmacokinetic parameters and their variability.

Inter-individual variability of the pharmacokinetic parameters was estimated using an exponential model and could be expressed as follows:

$$\theta_i = \theta_{TV} * e^{\eta^i}$$

where θ_i represents the parameter value of the i^{th} subject, θ_{TV} the typical value of the parameter in the population and η^i the variability between subjects which is assumed to follow a normal distribution with a mean of zero and variance ω^2 .

Covariate analysis followed a forward and backward selection process. The stepwise covariate modelling (17,18) and likelihood ratio test was used to test the effect of each variable. Model validation was based on graphical and statistical criteria, including goodness-of-fit plots (19), mirror plots, bootstrap, visual predictive check (VPC) and normalized prediction distribution errors (NPDE) (20,21).

Clinical application in therapeutic drug monitoring

Given our interest in clinical application of model-based approaches, the performance of the final model to support therapeutic drug monitoring and dosing adjustment was tested via simulation scenarios. To assess its predictive value, we have extensively evaluated whether the final model could be used to accurately predict observed drug exposure with current dosing regimens. For this purpose, the time course of abacavir concentrations was simulated 100 times in each sub-population (infants, toddlers and children) and for each dosing regimen (once vs. twice daily). The area under the concentration vs. time curve (AUC_{0-24}) was selected as endpoint for the purposes of this evaluation and AUC_{0-24} ($2 \times AUC_{0-12}$ for twice daily) was calculated using trapezoidal rule. The simulated AUC_{0-24} was then compared with median observed AUC_{0-24} .

The feasibility of a model-based approach in therapeutic drug monitoring was evaluated by considering two main scenarios in which pooled population data and sparse pharmacokinetic sampling are used as basis for predicting drug exposure in new patients:

1. To assess model performance in new patients, 10 children were randomly removed from the original dataset. The parameters for the remaining 59 children were re-estimated. The model parameters were then used to predict individually the pharmacokinetics of the 10 children excluded from the analysis, taking into account the effect of covariates in each patient. Predictions were compared to the observed data graphically by means of visual predictive check plots (1000 simulations/patient).

2. To assess the impact of empirical sparse sampling on model predictions, data from new patients using only three samples (T0, T1 and T3) were added in a stepwise manner to the dataset (i.e., initial population, n=59). Model parameters were then re-estimated for all 60 children (of which 59 had frequent sampling scheme). The new model was used to predict the full pharmacokinetic profile of single patients with sparse samples. Results were compared graphically with the original data using visual predictive check plots (1000 simulations/patient). This approach was selected as an initial step to the use of a full Bayesian analysis, in which model parameter values from a historical population (instead of the data) are used as priors to anchor the estimation of the parameters of interest for a new subject or population.

4.3. RESULTS

Population pharmacokinetic modelling

A total of 1065 plasma abacavir concentrations were available for population modelling. Data fitted a two-compartment model with first order absorption and elimination. Inter-individual variability was best described by an exponential model and was then estimated for Q/F , V_1/F , V_2/F and CL/F . Inter-occasion variability on CL/F was coupled to inter-individual variability by an additive model, respectively. Residual variability was best described by a proportional model.

Table 1: Summary of three pharmacokinetics studies and characteristics of patients

	Penta13	Penta 15	ARROW	Integrated analysis
SUBJECTS	14	18	37	69
STEADY STATE	Yes	Yes	Yes	Yes
MEDIAN AGE (years)	5.10	1.93	7.61	5.74
MIN (years)	2.14	0.42	3.62	0.42
MAX (years)	12.84	2.81	12.54	12.84
MEDIAN WEIGHT (kg)	19.2	11.6	20.5	17.6
MIN (kg)	14.0	7.6	14	7.6
MAX (kg)	60.9	15.8	29.8	60.9

During covariate model building, the inclusion of age, weight and formulation on CL/F, and weight on V_1/F all separately produced a significant decrease in objective function (OFV). However, following the backward exclusion process, only the effect of weight on CL/F and V_1/F was found to be significant ($(\Delta\text{OFV} > 7.88$ ($p < 0.005$, χ^2 distribution)). Therefore, the influence of weight on CL/F and V_1/F was retained in the model as follows:

$$\text{CL}/F_i = \text{CL}/F_{\text{ref}} \times (\text{WT}_i / \text{WT}_{\text{ref}})^{0.1}$$

$$\text{V}_1/F_i = \text{V}_1/F_{\text{ref}} \times (\text{WT}_i / \text{WT}_{\text{ref}})^{0.2}$$

Where CL/F_i and V_1/F_i are, respectively the CL/F and V_1/F of the i^{th} individual, WT_i the weight of the i^{th} individual, WT_{ref} the reference weight. The subscript “ref” indicates the individual with a reference weight. In our study, the reference weight was the median value of our population 17.6 kg. The allometric exponents were estimated to be 0.802 for CL/F and 0.810 for V_1/F .

Model diagnostics indicated acceptable goodness-of-fit for the final model. As shown in figure 1a, population and individual predictions are unbiased. In addition, the mean parameter estimates resulting from the bootstrap procedure very closely agreed with the respective values from the final population model, indicating that the estimates for the population pharmacokinetic parameters in the final model were accurate and that the model was stable. The results of 1000 bootstrap replicates are summarised in table 2.

Mirror plots reveal that the variance-covariance structure was well characterised, as the simulated datasets reproduce the similar dispersion pattern observed in the original data (results not shown). The NPDE distribution and histogram indicates that the assumption of normal distribution of the differences between individual predictions and observed data is acceptable (figure 1b). No trends were observed on the diagnostic plots of NPDE versus time. The VPC (figure 2) of the final model with all patients shows that observed concentrations were well predicted by the model (Exact Binomial Test, 7.4% out of limits observed, 95% confidence interval [5.9% – 9.2%]). VPCs for each sub-population (infants, toddlers and children) and each dosing regimen (once and twice daily) are also shown in figure 2.

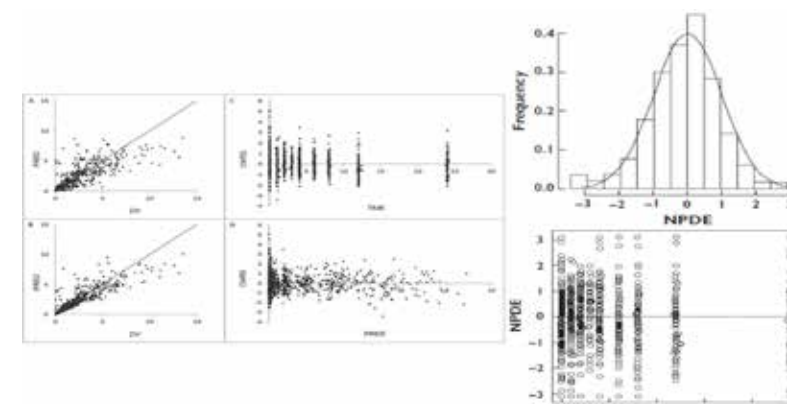


Figure 1 (a) Goodness-of-fit. Left upper panel shows the population prediction (PRED) vs. observed concentration values (DV). Right upper panel shows conditional weighted residuals (CWRES) vs. time (TIME). Left lower panel shows individual predictions (IPRE) vs. observed concentration values (DV). Right lower panel shows conditional weighted residuals (CWRES) vs. population predictions (PRED). Solid line represents the identity line. (b) Normalized Prediction Distribution Errors (NPDE) analysis. Upper panel shows the histogram of the distribution of the NPDE, with the density of the standard Gaussian distribution overlaid. Lower panel shows NPDE versus time (TIME)

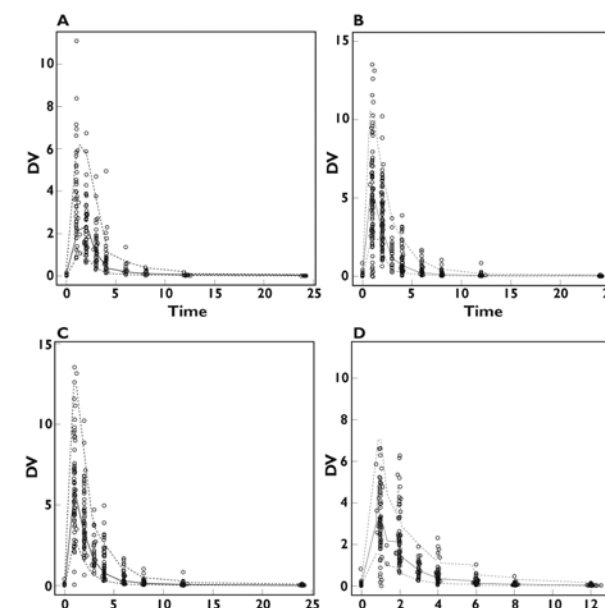


Figure 2 VPC in infants and toddlers (a), children (b), following once daily (c) and twice daily dosing regimen (d): observed data are plotted using a circle (\circ). The dashed lines represent the 5th, and 95th percentiles of simulated data ($n=1000$). The solid lines represent the 50th of simulated data ($n=1000$).

Table 2 Population pharmacokinetic parameters of abacavir and bootstrap validation

Parameter ¹	Popu- lation Estimate	%CV	Bootstrap Mean (95%CI)
Clearance (CL) CL/F= $\theta_1 \cdot (\text{BW}/\text{med})^{\theta_5}$ (intercept) L/h	20.1	3.8	20.1 (18.7-21.4)
(exponent) L/h/kg	0.802	11.6	0.796 (0.651- 0.954)
Inter-compartmental Clearance (Q) L h ⁻¹	2.0	9.9	2.0 (1.7-2.4)
Central Volume of distribution (V ₁) V ₁ /F= $\theta_2 \cdot (\text{BW}/\text{med})^{\theta_6}$ (intercept) L	13.0	11.7	12.8 (9.3-15.5)
(exponent) L/kg	0.810	23.3	0.793 (0.330- 0.954)
Peripheral volume of distribution (V ₂) L	13.5	10.7	2.0 (1.7-2.4)
Absorption rate constant (K _a) 1/h	0.913	4.c	0.909 (0.842- 0.985)
Interindividual variability			
$\eta_{\text{CL/F}}$ variance			
η_{Q} variance	42.5% ²	41.7	41.0 (27.5-62.1)
$\eta_{\text{V1/F}}$ variance	47.7% ²	33.6	46.2 (29.7-66.4)
$\eta_{\text{V2/F}}$ variance	57.5% ²	40.5	55.8 (38.3-76.4)
Inter-occasion variability			
OCCCL	20.4% ³	25.3	20.2 (15.7-24.8)
Residual Error			
Proportional error	38.2%	8.2	38.1 (35.7-40.9)

1. Population parameter point-estimates for the full two-compartment model and 95% CI and %CV from a non-parametric bootstrap are presented.
2. Value in parentheses represents the interindividual variability of the pharmacokinetic parameters calculated as the square root of $\Omega \times 100\%$
3. Value in parentheses represents the inter-occasion variability of the pharmacokinetic parameters calculated as the square root of the $\Omega \times 100\%$

Predictive performance in clinical applications

To assess the performance of the final model for therapeutic drug monitoring and dose adjustment, pharmacokinetic parameter estimates were also used to simulate drug exposure, expressed as area under the concentration vs. time curve AUC₀₋₂₄, in different sub-populations (infants and toddlers [n=21, age range: 0.42-2.81]; children [n=48, age range: 3.58-12.84]) and for currently used dosing regimens (once and twice daily dosing). As shown in figure 3, considerable overlap was observed in the simulated and observed AUC₀₋₂₄ values in infants and toddlers, and children. Model predicted Cmax and AUC₀₋₁₂ (geometric mean) of standard dose regimen (8mg/kg twice daily) were 2.5 mg/L and 6.1 mg•h/L in toddlers and infants, and 3.6 mg/L and 8.7 mg•h/L in children, respectively. These values were in agreement with the observed values in the original studies. In fact, the observed Cmax and AUC₀₋₁₂ (geometric mean) were respectively 2.3 mg/L and 5.8 mg•h/L in toddlers and infants, and 3.6 mg/L and 8.2 mg•h/L in children. Similarly, drug exposure was not different after once or twice daily doses of abacavir.

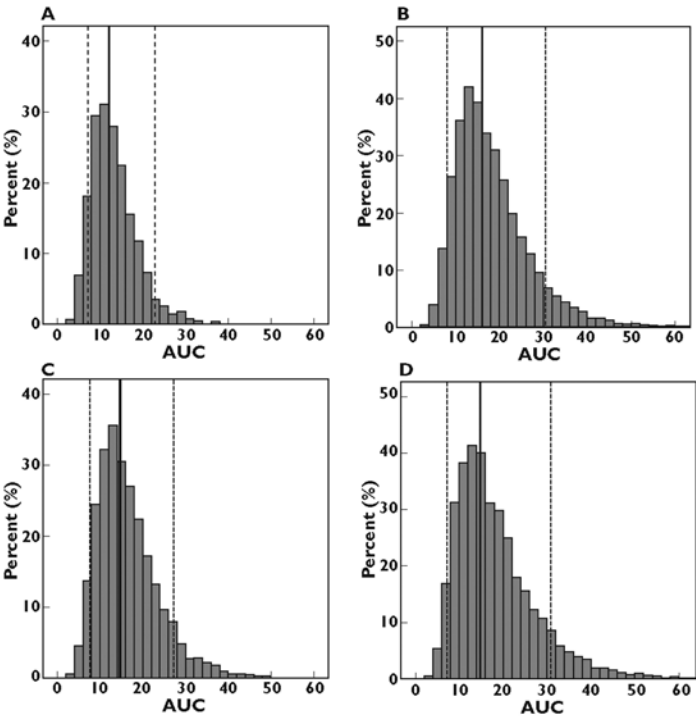


Figure 3 Simulated AUC distribution and median (continuous line) and 5th and 95th percentiles (dashed lines) of the observed AUC in infants and toddlers (a), in children (b), following once daily dosing (c) and twice daily dosing (d)

Moreover, the assessment of the predictive performance of the model included scenarios in which drug exposure was predicted in new patients taking sparse sampling schemes into account. In both cases, estimates of parameter accuracy and precision were acceptable. As shown in figure 4, accurate predictions can be made of individual patient profiles using this model, despite some evidence of over-estimation of residual variability.

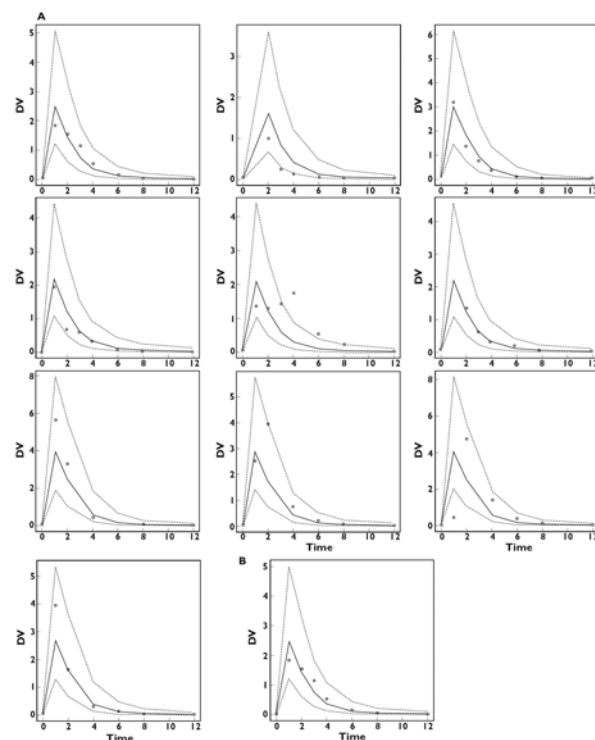


Figure 4 Individual VPC for new patients. Scenario (a): VPC for 10 new patients. Scenario (b): VPC for 1 patient with sparse sampling. Open circles (o) represent the observed data, whilst dashed lines depict the 5th and 95th percentiles of the simulated data ($n=1000$). The solid lines indicate the median obtained from the simulated data ($n=1000$).

4.4. DISCUSSIONS AND CONCLUSIONS

In the present study, we have shown the use of population pharmacokinetic meta-analysis of abacavir based on data obtained by a rich sampling strategy in 69 children from three pharmacokinetic studies. We believe that pooling of data offers the opportunity to evaluate drug disposition across a wide age and body weight range. Such an evaluation may be essential to assess the suitability of dosing recommendations for children. Even though our analysis is limited to abacavir data, we anticipate that such considerations are necessary and applicable to most if not all compounds for paediatric indications.

From a methodological perspective, meta-analytical concepts are required to ensure thorough understanding of the implications of developmental growth on pharmacokinetics in paediatric patients. Despite attempts to describe changes in drug disposition by allometric models, it should be clear that the paediatric population encompasses a very heterogeneous group of patients. Inferences about pharmacokinetics in individual patients may be challenging with data arising from a very limited number of patients, especially when the objective is to predict individual exposure in prospective patients or to adjust dosing regimens in chronic treatment, as in the case of therapeutic drug monitoring. The scope of population pharmacokinetic modelling is to enable the description and prediction of ADME processes in a parametric manner, so that hierarchical parameters can be derived that can discriminate population from individual patient characteristics. In paediatric pharmacokinetics, however, discrimination between population and individual differences is further confounded by the role of maturation and other factors associated with developmental growth, including changes in metabolic capacity (22). In a previous work (7), Cella *et al* have shown that a model-based approach offers a suitable basis for estimation of pharmacokinetic parameters even when only sparse samples may be available. However, such models do not necessarily permit accurate prediction of the differences in pharmacokinetics for individuals whose characteristics are not represented in the population used during model building and validation. As shown in a previous analysis (23), a model developed using data in older children cannot reliably predict exposure in infants and toddlers, and vice versa. This lack of predictive performance is partly explained by the fact that covariate-parameter correlations may not remain constant beyond the range of observations. Estimation of covariate effects is therefore not sufficient to allow accurate extrapolation of pharmacokinetics from a reference population to another population.

Our results indicate that it is not the overall number of patients that determines the predictive performance of a model, but rather the availability of data from the overall population, so that parameter distributions can be accurately estimated and imputations can be made about individuals belonging to any part of the population with adequate precision. Our results show that good predictive performance of a model can be achieved with a considerably limited number of individuals as long as the covariate distribution in the subjects used for model building represents

the covariate distribution in the population described by the model. This is critical to ensure that differences driven by covariates are not captured as random effects, nor random effects are wrongly associated with covariates. This is illustrated by the difference in the magnitude of parameter estimates in our analysis and in estimated parameters from single trials (table 3).

Table 3 Covariate-parameter relationships identified for abacavir in previous population pharmacokinetic analyses.

Study	Ref.	Number of children	Age range (years)	Significant covariates in the model	Covariate-parameter relationship
Penta 13	[7]	14	2.14-12.84	Weight on CL and V	CL/F (L/h) = 37.2 • {BW/23.8}0.553 V/F (L) = 64.8 • {BW/23.8}0.537
Penta 15	[12]	18	0.42-2.81	Weight on CL	CL/F (L/h) = 13.4 • {BW/12}1.14
Penta 13+Penta 15+ARROW	This article	69	0.42-12.84	Weight on CL and V1	CL/F (L/h) = 20.1 • {BW/17.6}0.802 V1/F (L) = 13.0 • {BW/17.6}0.810
Therapeutic drug monitoring data	[11]	105	0.0685-16	Weight on CL and V	CL/F (L/h) = 24.3 • {BW/25}1.0 V/F (L) = 42.9 • {BW/25}0.95.

Whereas the focus of previous publications was on the use of modelling as the basis for drug development (i.e., early paediatric trials), little attention has been paid to the implications of similar modelling requirements for accurate dosing adjustment and therapeutic drug monitoring in clinical practice (24, 25). In the present study, we have assessed the predictive performances of the final model using several simulation scenarios in which potential differences in individual exposure are evaluated. Our results indicated that the final model can accurately predict drug exposure with currently used dosing regimens in new patients, even in case of sparse sampling.

Population pharmacokinetic and/or pharmacodynamic model validation is another key- issue to consider when models are to be used for simulation purposes (i.e. dosage optimisation or clinical trial simulation). Validation procedures are lacking in many publications reporting the development of population pharmacokinetic and /or pharmacodynamic models (26). In fact, advanced internal evaluations were performed on merely 16% of the models in children (27). In the present study, five evaluation / validation criteria were included: 1) Standard goodness-of-fit plots, which inform on model misspecification and allow assessment of trends or bias in the model predictions. 2) Mirror plots, which allow comparison of the variance structure between simulated and observed data. 3) Bootstrap, which provides information on the stability of the final model. A robust model is not affected by the contribution or influence of specific individuals in the data set. 4) Visual Predictive check, which yields information on the presence of systemic

bias or deviations (trends) in model predictions. 5) NPDE, which provides details on the distribution of the differences between predictions and observations. It is an important criterion for the validation of a model for subsequent simulation purposes. Even though each of the aforementioned diagnostic tools reveals different aspects of model performance, it is critical to point out that there is no guarantee that model predictions will be accurate unless the relevant covariates are included in the initial model.

Limitations

During this investigation only body weight, age, gender and formulation were tested as potentially influential covariates on pharmacokinetic parameters. Information on ethnicity and other potential demographic factors were not available. Given that abacavir is metabolised primarily through alcohol dehydrogenase or glucuronyl transferase, metabolic information would have been useful to describe abacavir pharmacokinetics. Further studies are required to evaluate the ontogeny of abacavir metabolism.

In summary, we have shown that abacavir pharmacokinetics in children can be characterised by a two-compartment model with first order absorption. Body weight was identified as the primary covariate influencing the apparent oral clearance and volume of distribution. The availability of data across a wide range of ages and consequently across body weights enabled the identification of the accurate relationships between pharmacokinetic parameters and covariates in the paediatric population. These relationships may not be evident or even missed when analysing small datasets or when the relevant range of values for the influential covariates is not included in the overall population. The use of an integrated, meta-analytical approach is therefore essential to ensure accurate prediction of drug exposure in new patients or in clinical conditions different from the original trial setting.

References

1. Antiretroviral therapy for HIV infection in infants and children. Available at <http://www.who.int/hiv/pub/paediatric/paed-prelim-summary.pdf> (last accessed 18 May 2013)
2. PENTA Steering Committee, Welch S, Sharland M, Lyall EG, Tudor-Williams G, Niehues T, Wintergerst U, Bunupuradah T, Hainaut M, Della Negra M, Pena MJ, Amador JT, Gattinara GC, Compagnucci A, Faye A, Giaquinto C, Gibb DM, Gandhi K, Forcat S, Buckberry K, Harper L, Königs C, Patel D, Bastiaans D. PENTA 2009 guidelines for the use of antiretroviral therapy in paediatric HIV-1 infection. *HIV Medicine*; **10**: 591-613 (2009).
3. McDowell JA, Chittick GE, Ravitch JR, Polk RE, Kerkering TM, Stein DS. Pharmacokinetics of [(14)C] abacavir, a human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitor, administered in a single oral dose to HIV-1-infected adults: a mass balance study. *Antimicrobial agents and chemotherapy* **43**, 2855-61 (1999).
4. Yuen GJ, Weller S, Pakes GE. A review of the pharmacokinetics of abacavir. *Clinical pharmacokinetics* **47**, 351-71 (2008).
5. Faletto MB, Miller WH, Garvey EP, St Clair MH, Daluge SM, Good SS. Unique intracellular activation of the potent anti-human immunodeficiency virus agent 1592U89. *Antimicrobial agents and chemotherapy* **41**, 1099-107 (1997).
6. Weller S, Radomski KM, Lou Y, Stein DS. Population pharmacokinetics and pharmacodynamic modelling of abacavir (1592U89) from a dose-ranging, double-blind, randomized monotherapy trial with human immunodeficiency virus-infected subjects. *Antimicrobial agents and chemotherapy* **44**, 2052-60 (2000).
7. Cella M, Gorter de Vries F, Burger D, Danhof M, Della Pasqua O. A model-based approach to dose selection in early pediatric development. *Clinical pharmacology and therapeutics* **87**, 294-302 (2010).
8. Bergshoeff A, Burger D, Verweij C, Farrelly L, Flynn J, Le Prevost M, Walker S, Novelli V, Lyall H, Khoo S, Gibb D; PENTA-13 Study Group. Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
9. Paediatric European Network for Treatment of AIDS (PENTA). Pharmacokinetic study of once-daily versus twice-daily abacavir and lamivudine in HIV type-1-infected children aged 3-<36 months. *Antiviral therapy* **15**, 297-305 (2010).
10. Musiime V, Kendall L, Bakeera-Kitaka S, Snowden WB, Odongo F, Thomason M, Musoke P, Adkison K, Burger D, Mugenyi P, Kekitiinwa A, Gibb DM, Walker AS; ARROW Trial team. Pharmacokinetics and acceptability of once- versus twice-daily lamivudine and abacavir in HIV type-1-infected Ugandan children in the ARROW Trial. *Antiviral therapy* **15**, 1115-24 (2010).
11. Jullien V, Urien S, Chappuy H, Dimet J, Rey E, Pons G, Blanche S, Tréluyer JM. Abacavir pharmacokinetics in human immunodeficiency virus-infected children ranging in age from 1 month to 16 years: a population analysis. *Journal of clinical pharmacology* **45**, 257-64 (2005).
12. Zhao W, Cella M, Della Pasqua O, Burger D, Jacqz-Aigrain E, on behalf of Pediatric European Network for Treatment of AIDS (PENTA) 15 study group. Population pharmacokinetics and Bayesian estimator of abacavir: application of individualized therapy in HIV infected infants and toddlers. *British journal of clinical pharmacology* **73**, 641-50 (2012).
13. Kline MW, Blanchard S, Fletcher CV, Shenep JL, McKinney RE Jr, Brundage RC, Culnane M, Van Dyke RB, Dankner WM, Kovacs A, McDowell JA, Hetherington S. A phase I study of abacavir (1592U89) alone and in combination with other antiretroviral agents in infants and children with human immunodeficiency virus infection. AIDS Clinical Trials Group 330 Team. *Pediatrics* **103**, e47 (1999).
14. Hughes W, McDowell JA, Shenep J, Flynn P, Kline MW, Yogev R, Symonds W, Lou Y, Hetherington S. Safety and single-dose pharmacokinetics of abacavir (1592U89) in human immunodeficiency virus type 1-infected children. *Antimicrob agents and chemotherapy* **43**, 609-15 (1999).
15. Cross SJ, Rodman JH, Lindsey JC, Robbins BL, Rose CH, Yuen GJ, D'angelo LJ. Abacavir and metabolite pharmacokinetics in HIV-1-infected children and adolescents. *Journal of acquired immune deficiency syndrome* **51**, 54-9 (2009).
16. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. *Computational methods and programs in biomedicine* **75**, 85-94 (2004).
17. De Cock RF, Piana C, Krekels EH, Danhof M, Allegaert K, Knibbe CA. The role of population PK-PD modelling in paediatric clinical research. *European journal of clinical pharmacology* **67**, 5-16 (2011).
18. Ribbing J, Jonsson EN. Power, selection bias and predictive performance of the Population Pharmacokinetic Covariate Model. *Journal of pharmacokinetics and pharmacodynamics*; **31**, 109-34 (2004).
19. Hooker AC, Staats CE, Karlsson MO. Conditional weighted residuals (CWRES): a model diagnostic for the FOCE method. *Pharmaceutical research* **24**, 2187-97 (2007).

- 20 Brendel K, Comets E, Laffont C, Laveille C, Mentré F. Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. *Pharmaceutical research* **23**, 2036-49 (2006).
- 21 Comets E, Brendel K, Mentré F. Computing normalised prediction distribution errors to evaluate non-linear mixed-effect models: the npde add-on package for R. *Computational methods and programs in biomedicine* **90**, 154-66 (2008).
- 22 Cella M, Knibbe C, Danhof M, Pasqua OD. What is the right dose for children? *British journal of clinical pharmacology* **70**, 597-603 (2010).
- 23 Cella M, Zhao W, Jacqz-Aigrain E, Burger D, Danhof M, Pasqua OD. Paediatric drug development: are population models predictive of pharmacokinetics across paediatric populations? *British journal of clinical pharmacology* **72**, 454-64 (2011).
- 24 Neely M, Jelliffe R. Practical therapeutic drug management in HIV-infected patients: use of population pharmacokinetic models supplemented by individualized Bayesian dose optimization. *Journal of clinical pharmacology* **48**, 1081-91 (2008).
- 25 Zhao W, Fakhoury M, Fila M, Baudouin V, Deschênes G, Jacqz-Aigrain E. Individualization of valganciclovir prophylaxis for cytomegalovirus infection in pediatric kidney transplant patients. *Therapeutic drug monitoring* **34**, 326-30 (2012).
- 26 Brendel K, Dartois C, Comets E, Lemenuel-Diot A, Laveille C, Tranchand B, Girard P, Laffont CM, Mentré F. Are population pharmacokinetic and/or pharmacodynamic models adequately evaluated? A survey of the literature from 2002 to 2004. *Clinical pharmacokinetics* **46**, 221-34 (2007).
- 27 Tod M, Jullien V, Pons G. Facilitation of drug evaluation in children by population methods and modeling. *Clinical pharmacokinetics* **47**, 231-43 (2008).

INFLUENCE OF COVARIATE DISTRIBUTION ON MODEL PREDICTIVE PERFORMANCE

CHIARA PIANA, MEINDERT DANHOF, OSCAR DELLA PASQUA

Submitted for publication

SUMMARY

The accuracy of model-based predictions often reported in paediatric research has not been thoroughly characterised. The aim of this exercise is therefore to evaluate the role of covariate distributions when a pharmacokinetic model is used for simulation purposes. Of interest is the evidence that model predictions are unbiased when the covariate range is beyond the data distribution available during model-building. Such an analysis is critical for the use of nonlinear hierarchical modelling in extrapolation and bridging of findings across different populations in paediatric drug development.

Plasma concentrations of a hypothetical drug and demographic characteristics of a paediatric population were simulated using a pharmacokinetic model in which body weight was correlated to clearance and volume of distribution. Two subgroups of children were then selected from the overall population according to a typical study design, in which pre-specified body weight ranges (10-15 kg and 30-40 kg) are used as inclusion criteria. The simulated data sets were then analysed using non-linear mixed effects modelling. Model performance was assessed by comparing the accuracy of AUC predictions obtained for each subgroup, based on the model derived from the overall population and by extrapolation of the model parameters across subgroups.

Our findings show that systemic exposure as well as pharmacokinetic parameters (CL and V) cannot be accurately predicted from the pharmacokinetic model obtained from a population with a different covariate range than the one explored during model building. Predictions were accurate only when a model is used for prediction in a subgroup of the initial population. In contrast to current practice, the use of pharmacokinetic modelling in children should be limited to interpolations within the range of values observed during model building. Furthermore, the covariate point estimate must be kept in the model even when predictions refer to a subset different from the original population. These findings highlight the importance of meta-analytical procedures in paediatric bridging. It also suggests the need for more mechanistic parameterisations, which discriminate drug from system-specific parameters.

5.1. INTRODUCTION

Modelling and simulation (M&S) of clinical data represents a powerful approach for evidence synthesis and consequently for a more comprehensive interpretation of the data available at any point in time during the process of drug development. Ideally, it should also provide the basis for inferences and extrapolation of findings from a subgroup to the entire target population (1, 2). At present, regulatory bodies in Europe encourage the application of the M&S approach during drug development (3), as it may circumvent some practical and ethical difficulties in the evaluation of paediatric medicines. In fact, industry and academia have been developing and applying models under the assumption that nonlinear mixed effects modelling methods are robust enough to enable the characterisation of pharmacokinetics and pharmacodynamics even when sparse sampling and unbalanced data sets are used (4–8).

More recently, M&S has been applied as a design tool for pharmacokinetic and pharmacokinetic-pharmacodynamic bridging. In this case, the main objectives of such models include the selection of dose and prediction of drug exposure and/or effects in a new population, for which no data has been generated. In this context, the identification of influential covariates such as demographic characteristics can play a major role in the accuracy of parameter estimates and subsequent predictions. The assessment of the correct correlations between covariates and parameters is crucial, given that it will have direct implication for the dose selection in a new population with different demographic characteristics. In the paediatric research, however, the identification of the correct covariates is often complex due to the presence of correlations and co-linearity between covariates. As shown by a previous investigation from Ribbing *et al*, competition between multiple covariates may further increase selection bias, especially when there is a moderate to high correlation between the covariates (9).

Different methods are available to select significant covariates during model building. The most used one is the stepwise covariate selection in which two processes, forward inclusion and backward elimination, are applied (10,11). Alternative methods, such as genetic algorithms for covariate selection (12) and automated covariate model building (13, 14) are also becoming more common, but have not been scrutinised to the same extent in pharmacokinetic research. In contrast to traditional data analysis, models developed by the stepwise covariate selection are also being used to predict drug exposure and consequently define the dose rationale in new patients, whose characteristics differ from the original patients in the trial.

Model-based predictions can be considered for a population with similar characteristics as the one under investigation during model building (interpolation) or for a new population beyond the covariate range explored during model building (extrapolation). Many examples are available in literature of studies in which modelling has been applied to interpolate data (15–17). Fewer examples exist however in which extrapolations are made to a population which does not share exactly the same characteristics or includes individuals beyond the range of

values explored during model building. In fact, two recent publications by Cella *et al*. (18, 19) showed the lack of predictive performance of pharmacokinetic models when they are used for extrapolation purposes.

The current analysis has two main objectives. First of all, we want to define the feasibility and pre-requisites to use pharmacokinetic models as an extrapolation tool, i.e., to make predictions about a population in which the values of the covariates are beyond the covariate ranges explored during model building. Secondly, we want to investigate how the parameter-covariate correlation needs to be expressed when a model is used for simulation purposes. From a methodological perspective, there are different ways to express the correlation parameter-covariate during covariate selection. Among other options, as shown in equations 1 and 2, we evaluate the impact of “centring” on the median or the mean value of the covariate in the population. This approach is supposed to stabilise parameter estimation and facilitate the interpretation of the coefficients in the correlation.

$$P = \theta_1 + \theta_2 * COV \quad (1)$$

$$P = \theta_1 + \theta_2 * (COV / COV_{median}) \quad (2)$$

In these equations P is the parameter, COV is the covariate, COVmedian the median value of the covariate in the dataset and θ_1 and θ_2 are the slope and the intercept which describes the correlation between the parameter and the covariate, respectively. In both equations the intercept and the slope are estimated during model building. In the first case (equation (1)), the variation in the values of the covariate can cause instability in the estimation of slope and intercept. This contrasts with the second case (equation (2)) as the intercept is roughly centred; this means, for instance, that for adult populations 70 is commonly used as a median value of body weight.

In this study, in addition to investigating the predictive performance of a pharmacokinetic model for extrapolation purposes, we evaluate whether the covariate point estimate must be retained in the model when extrapolations refer to a population in which the median or mean value differ from the one in the population previously analysed or whether it should be adapted to reflect the covariate distribution of the new population.

5.2. METHODS

Population demographics and hypothetical protocol

A group of 43 virtual paediatric patients with a weight range between 7.43 and 61.3 kg (median weight 14.2 kg) were sampled from a pooled dataset including demographic characteristics from three pharmacokinetic studies (20–22). The sampling procedure was performed in such a way that the age and body weight distribution in the virtual population was balanced across the weight range of interest.

These patients were then treated with a hypothetical drug, given orally every 12 hours. A total of 8 plasma samples per subject were then simulated throughout the dosing interval. Using data from the overall population (group C), two subgroups were selected based on different body weight range. As shown in table 1, the first subgroup (subgroup A) comprised 20 children with weight between 10.3 and 15.4 kg (median body weight 12.5 kg), whilst the second group (subgroup B) included 8 children with weight between 30 and 43.8 kg (median body weight 35 kg).

Table 1 Summary of demographic characteristics of the hypothetical population.

	SUBGROUP A	SUBGROUP B	GROUP C (Full population)
Subjects	20	8	43
Median weight (kg)	12.5	35.05	14.2
Min weight (kg)	10.3	30.05	7.43
Max weight (kg)	15.4	43.8	61.3
Median age (years)	2.18	8.85	2.81
Min age (years)	0.99	8.1	0.42
Max age (years)	3.89	12.67	12.92

Predefined covariate effects

The pharmacokinetics of the compound was assumed to be described by a one compartment pharmacokinetic model with first-order absorption and elimination. A base model previously developed by our group for lamivudine (Covariate effects and population pharmacokinetics of lamivudine in HIV-infected children-*In press, British Journal of Clinical Pharmacology*) was used for simulations.

Various scenarios were simulated, in which body weight was linearly and/or exponentially correlated to clearance and volume of distribution. Allometric scaling concepts were also taken into account, but the exponents were explored with values higher and lower than 0.75. Four different scenarios in which one covariate was significant were simulated, based on realistic parameter-covariate correlations:

- Body weight linearly correlated with clearance (with a slope of 0.65)
- Body weight linearly correlated with clearance (with a slope of 1.5)
- Body weight exponentially correlated with clearance (with an exponent of 0.65)
- Body weight exponentially correlated with clearance (with an exponent of 1.5)

Given that usually more than one covariate is found to influence pharmacokinetic parameters, two additional scenarios were simulated in which a second covariate was incorporated into the model.

- Body weight linearly correlated with volume of distribution (with a slope of 1.8) (15) and exponentially correlated with clearance (with an exponent of 0.65)
- Body weight exponentially correlated with volume of distribution (with an exponent of 0.635) and exponentially correlated with clearance (with an exponent of 0.705) (Covariate effects and population pharmacokinetics of lamivudine in HIV-infected children -*In press British Journal of Clinical Pharmacology*)

The relationship between parameter and covariate was described as follows:

$$P=\theta_1 + (WT/WT_{Median})*\theta_2 \tag{3}$$

$$P=\theta_1 *(WT/WT_{Median})^{**}\theta_2 \tag{4}$$

In these equations P is the pharmacokinetic parameter (in this case clearance or volume of distribution), WT is body weight and WT_{median} is the median of the body weight distribution in the population analysed during model building. Equation (3) represents a linear relationship between the parameter and body weight with θ_1 and θ_2 as the intercept and the slope of the correlation, respectively. Equation (4) represents an exponential relationship between the parameter and body weight with θ_1 and θ_2 as the coefficient and the exponent of the correlation, respectively.

Analysis of simulated patient data: pharmacokinetic model and covariate criteria

The simulated plasma concentration datasets (full population and subgroup A) were subsequently fitted to a pharmacokinetic model according to the standard model building criteria. Body weight and age were tested according to a stepwise covariate inclusion approach (23), i.e., the covariates were entered one by one into the population model. After all significant covariates have been entered into the model (forward selection), each covariate was removed (backward elimination), one at a time. The likelihood ratio test was used to assess whether the difference in the objective function between the base model and the full (more complex) model was significant.

Additional parameters leading to a decrease in the objective function ≥ 3.84 were considered significant ($p < 0.05$). During the final steps of the model building, only the covariates which resulted in a difference of objective function ≥ 7.88 ($p < 0.005$) were kept in the final model. Each model was internally validated using a visual predictive check.

Model predictive performance: Posterior Predictive Check (PPC)

In order to evaluate model performance, a posterior predictive check (PPC) was carried out. PPC operates under the basic assumption that, if the model provides an adequate description of the data, then the simulated data from the same model should mimic the essential features of the observed data. In this investigation, model performance was assessed by comparing the accuracy of area under the curve (AUC) estimates obtained for each subgroup, based 1) on a model derived from the overall population and 2) by extrapolations to subgroup B ($n=8$), based on a model derived from subgroup A ($n=20$). This was done for each scenario, as described previously. AUC estimation was performed by keeping the median body weight of the population analysed during model building or by adapting the parameter correlation using the body weight median of the new population. Integration of the concentration time data was performed according to the trapezoidal rule.

5.3. RESULTS

In this paper, we summarise the results from two out of the six simulation scenarios that were evaluated. These scenarios are representative of the whole investigation, in that they capture the key issues regarding covariate model building and the use of model-based approaches for dose selection or extrapolation of pharmacokinetics across populations. In particular, we focus on the scenario in which body weight is exponentially correlated with clearance according to an exponent higher or lower than 0.75. As shown in figure 1, each pharmacokinetic model was validated using a visual predictive check. Both the models obtained from the full population (group C) and from the subgroup of children weighting between 10 and 15 kg (subgroup A) seem to accurately predict the observations.

In figure 2 predictions of the AUC in the children from subgroup B are derived from the model built with the data from subgroup A. Clearly, the model does not accurately predict the parameter of interest when the covariate range in the new population differs from the one of the original model. Moreover, as shown on the right panel of figure 2, adjusting the median of body weight to the distribution of the new population did not result in any improvement in model performance.

In contrast to the aforementioned results, accurate predictions of the pharmacokinetic parameter of interest are obtained in each of the subgroups when using the model obtained by fitting

the full population data set (figure 2 and figure 3). In addition, the model seems to perform well if the covariate point estimate is kept in the model (left panels). These results also show that predictions are accurate only when a model is used for interpolation purposes, i.e., when predictions encompass the range of covariate values included in the model building. Interestingly, the model does not perform accurately anymore when the relation between clearance and body weight is adapted to reflect the covariate distribution in the new population. This happens irrespectively of the magnitude of the exponent which correlates body weight to clearance.

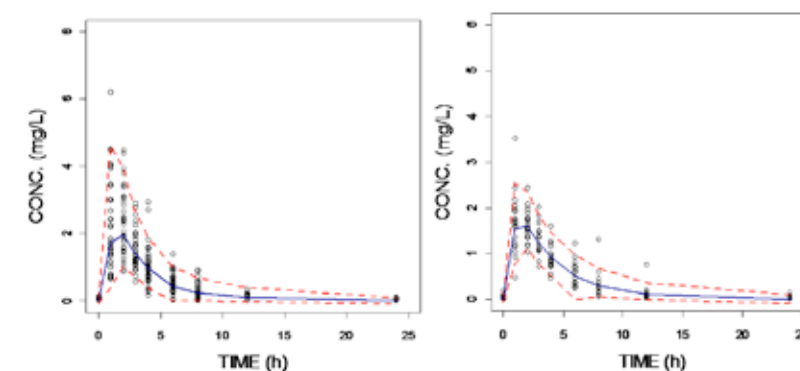


Figure 1 Visual predictive check of the models obtained from the fit of the simulated plasma concentrations of the children in subgroup A (right) and C (left) when body weight was exponentially correlated to clearance with an exponent of 0.65.

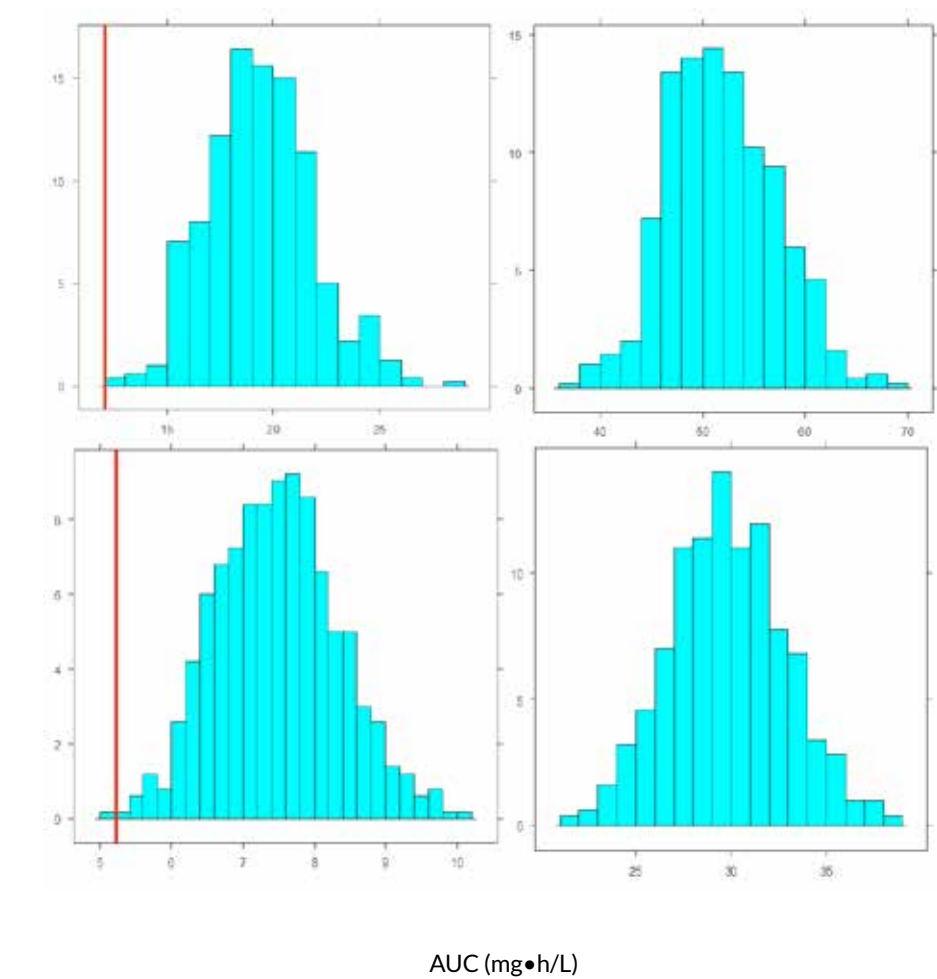


Figure 2 Predicted AUC distribution in subgroup B based on model parameter estimates obtained from data fitting of subgroup A. Upper panels show prediction distributions for an exponential correlation between clearance and body weight with an exponent of 0.65, whilst lower panels show prediction distributions for an exponent of 1.5. The line represents the true point estimate of AUC in the population. In the left panels the difference in the covariate distribution between subgroups A and B is not taken into account, with the median of the weight distribution in subgroup A being used in the simulations. In the right panels a shift is observed in the predictions when the covariate range of subgroup B is used in the simulations.

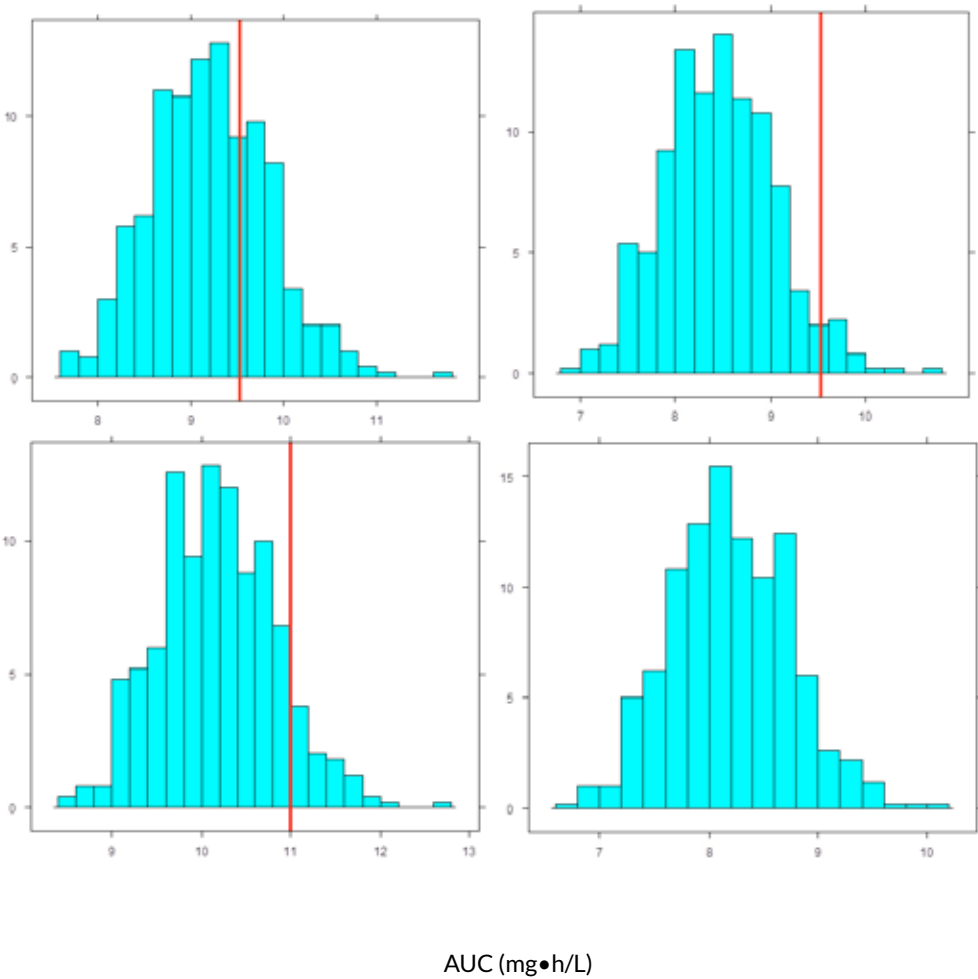


Figure 3 Predicted AUC distribution in subgroup A based on model parameter estimates obtained from data fitting of the full population (group C). Upper panels show prediction distributions for an exponential correlation between clearance and body weight with an exponent of 0.65, whilst the lower panels show prediction distributions for an exponent of 1.5. The line represents the true value of AUC in the population. In the left panels the difference in the covariate distribution between group C and subgroup A is not taken into account, with the median of the weight distribution of subgroup C being used in the simulations. In the right panels a shift is observed in the predictions when the covariate range of subgroup A is used in the simulations.

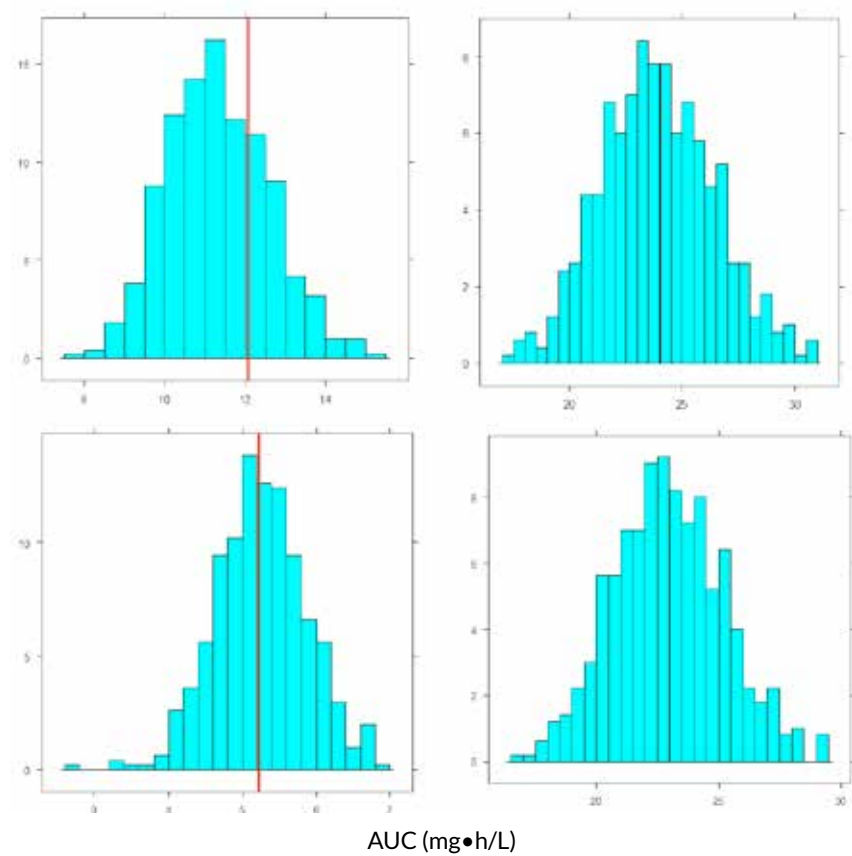


Figure 4 Predicted AUC distribution in subgroup B based on model parameter estimates obtained from data fitting of the full population (group C). Upper panels show prediction distributions for an exponential correlation between clearance and body weight with an exponent of 0.65, whilst the lower panels show prediction distributions for an exponent of 1.5. The line represents the true value of AUC in the population. In the left panels the difference in the covariate distribution between group C and subgroup B is not taken into account, with the median of the weight distribution of subgroup C being used in the simulations. In the right panels a shift is observed in the predictions when the covariate range of subgroup B is used in the simulations.

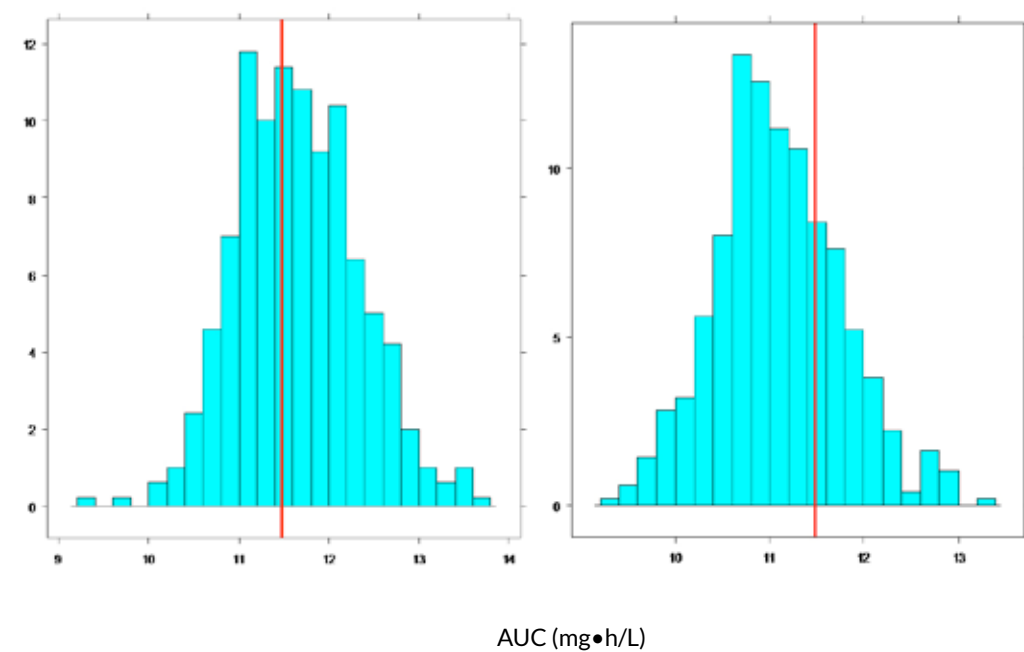


Figure 5 Predicted AUC distribution in subgroup B based on the model parameter estimates obtained from data fitting of subgroup A. The histograms show AUC predictions for a linear relation between clearance and body weight with a slope of 1.5. The line represents the true value of AUC in the population. In the left panel the difference in the covariate distribution between subgroups A and B is not taken into account, with the median of the weight distribution of subgroup A being used in the simulations. In the right panels a shift is observed in the predictions when the covariate range of subgroup B is used in the simulations.

5.4. DISCUSSIONS

The main focus of our study was to investigate the role of demographic covariates during bridging and extrapolation of pharmacokinetic data across paediatric populations. Clearly, the identification of influential covariate effects on pharmacokinetic parameters is crucial to ensure accurate dose selection or dose adjustment in a new population. This is particularly important during the planning phase of a bridging exercise, when pharmacokinetic models are used for simulation purposes. Ideally, the predictive performance of population models should be warranted before its application in paediatric therapeutic research and drug development. Here we have shown the potential for bias in model predictions when extrapolating data beyond the covariate range explored during model building, a common practice in industry and academic research, which relies in small sample sizes for the characterisation of pharmacokinetic properties of a compound. These findings emphasise the importance of meta-analysis and other techniques for evidence synthesis as the basis for any quantitative evaluation of pharmacokinetics and pharmacodynamics in children. From a methodological point of view we have also shown the relevance of “centring” on the point estimate of the covariate distribution, which must be retained in the model when extrapolations are performed, irrespectively of the differences in the covariate distribution in the population or subgroup of interest.

Model-based extrapolation and interpolation

The current findings show that extrapolation to a new population beyond the covariate range explored during model building is not possible for exponential parameter-covariate correlations. These results appear to be in agreement with a previous publication which showed that, irrespectively of whether extrapolation methods are to be applied during paediatric drug development, model predictions beyond the range of the data used for parameter estimation may be biased (18, 19). Adaptations or adjustments of parameter-covariate correlations to account for the covariate range of the new population does not improve model predictive performance. In fact, it appears that the farther the median of the covariate of the new population deviates from the original one, the less accurate is the predicted AUC distribution (right panels, figure 2). The only scenario which appears to yield accurate extrapolation from one group to another with different covariate values is when a linear correlation is used to describe the covariate effects (as shown in figure 5).

The (often physiologically-driven) exponential correlation between pharmacokinetic parameter and covariate is linked to nonlinearities that cannot be predicted without a semi- or fully mechanistic approach to bridging or extrapolations beyond the evidence derived from the available data. From a statistical perspective, this issue could be handled by defining uncertainty in parameter estimation.

However this would lead to wide parameter distributions, with little value for dosing recom-

mendation purposes. It should also be noted that this bias cannot be eliminated by the identification of additional covariates. Extrapolation to a different population requires accurate estimation of the underlying parameter-covariate correlations, which in turn imposes the availability of data (likelihood) or alternatively, the use of priors that support inferences about the parameter distribution in a different population, including the magnitude and nature of the covariate effects in those conditions. At present, our findings suggest that only interpolation is feasible when making use of nonlinear hierarchical models to describe pharmacokinetics in children. Interpolations will be accurate independently of the nature of the parameter-covariate correlations.

Influence of sample size on predictive performance

In addition to the hurdles for the use of bridging and extrapolation across populations, another issue in the covariate analysis presented here was the limited sample size of the data available for model building, which may clearly lead to wrong covariates selection and inclusion bias. In our study, the correct covariate (body weight) was identified when the full dataset was analysed, whilst a confounding factor (age) or no covariate effects (results not shown) were identified when evaluating the small, imbalanced subgroup of children ($n=20$). This problem, previously highlighted by Ribbing in his simulation study (9), emphasises the importance to perform a stepwise covariate selection only when large paediatric datasets are available. If this is not the case, meta-analysis or different methods, such as the use of a genetic algorithm in covariate selection (12) or automated covariate model building (13), should be considered.

Recommendations on the use of models for simulation purposes

M&S represents a powerful tool to avoid unnecessary studies in the target population as well as facilitate the interpretation of the limited evidence available (24). However, our findings underscore the importance of a careful and cautious use of models. Awareness about model assumptions and formal evaluation of the predictive performance of a model is required to avoid biased predictions, which in turn, could lead to wrong dosing adjustments in clinical practice.

The main recommendations from this investigation are listed below:

- Unless a mechanism-based model can be warranted, the use of a stepwise approach for covariate analysis is not recommended when small datasets are available. Instead, alternative approaches should be considered for paediatric bridging and extrapolation
- Extrapolation of the covariate effects beyond the parameter distributions explored during model building cannot be performed without bias, and consequently erroneous dosing recommendations.
- Pharmacokinetic models can be used for simulation purposes only when the population of interest can be considered a subgroup of the initial population.

- The covariate point estimate must be retained in the model when predictions refer to a population in which median or mean values differ from the population used during model building.

Limitations

Two main limitations need to be acknowledged in our study. First, we have restricted the analysis to a fixed number of virtual patients. This choice was based on the need to assess whether the dataset size may influence the final results. Therefore the same bioavailability was assumed in the whole population of children. We also acknowledge that from a methodological perspective, other algorithms could have been tested to confirm that these findings are not an artefact of the maximum likelihood, as implemented in NONMEM. The use of a different method, such as the stochastic approximation of the standard expectation maximization (SAEM) might have yielded different results in terms of the magnitude of the bias seen with the first-order conditional estimation method with interaction (FOCEI) (25)

Conclusions

In summary, model performance appears to be independent of the nature of the parameter-covariate correlations if predictions are restricted to interpolations. Biased results may be observed when predictions are aimed at extrapolations, i.e. covariate distribution lies outside the range observed during model building. The use of meta-analysis, mechanistic models and other alternative methods in which prior or historical data are used for inferential purposes is therefore recommended for bridging and extrapolation of pharmacokinetic data across paediatric populations. In addition, parameterisation of covariate effects based on the point estimate of the covariate distribution imposes the use of the same point estimate even when the population to be simulated differs from the original population.

These findings emphasise the need to discriminate between models for estimation and models for simulation, which are required when performing bridging and extrapolations. The discrepancies observed in the predicted distributions are not detectable with standard diagnostic tools currently used during model validation procedures.

References

1. Breimer, D.D. PK/PD modelling and beyond: impact on drug development. *Pharmaceutical research* **25**, 2720-2 (2008).
2. Bellanti, F. & Della Pasqua, O. Modelling and simulation as research tools in paediatric drug development. *European journal of clinical pharmacology* **67 Suppl 1**, 75-86 (2011).
3. Jönsson, S., Henningsson, A., Edholm, M. & Salmonson, T. Role of modelling and simulation: a European regulatory perspective. *Clinical pharmacokinetics* **51**, 69-76 (2012).
4. Manolis, E. & Pons, G. Proposals for model-based paediatric medicinal development within the current European Union regulatory framework. *British journal of clinical pharmacology* **68**, 493-501 (2009).
5. De Cock, R.F.W., Piana, C., Krekels, E.H.J., Danhof, M., Allegaert, K. & Knibbe, C.A.J. The role of population PK-PD modelling in paediatric clinical research. *European journal of clinical pharmacology* **67 Suppl 1**, 5-16 (2011).
6. Della Pasqua, O. & Rose, K. *Study and protocol design for paediatric patients of different ages. Guide to Paediatric Clinical Research* (2007).
7. Howie, S.R.C. Blood sample volumes in child health research: review of safe limits. *Bulletin of the World Health Organization* **89**, 46-53 (2011).
8. Meibohm, B., Läer, S., Panetta, J.C. & Barrett, J.S. Population pharmacokinetic studies in pediatrics: issues in design and analysis. *The AAPS journal* **7**, E475-87 (2005).
9. Ribbing, J. & Jonsson, E.N. Power, selection bias and predictive performance of the Population Pharmacokinetic Covariate Model. *Journal of pharmacokinetics and pharmacodynamics* **31**, 109-34 (2004).
10. Dartois, C. *et al.* Overview of model-building strategies in population PK/PD analyses: 2002-2004 literature survey. *British journal of clinical pharmacology* **64**, 603-12 (2007).
11. Wählby, U., Jonsson, E.N. & Karlsson, M.O. Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. *AAPS pharmSci* **4**, E27 (2002).

12. Bies, R.R., Muldoon, M.F., Pollock, B.G., Manuck, S., Smith, G. & Sale, M.E. A genetic algorithm-based, hybrid machine learning approach to model selection. *Journal of pharmacokinetics and pharmacodynamics* **33**, 195-221 (2006).
13. Lunn, D.J. Automated covariate selection and Bayesian model averaging in population PK/PD models. *Journal of pharmacokinetics and pharmacodynamics* **35**, 85-100 (2008).
14. Khandelwal, A., Harling, K., Jonsson, E.N., Hooker, A.C. & Karlsson, M.O. A fast method for testing covariates in population PK/PD Models. *The AAPS journal* **13**, 464-72 (2011).
15. Knibbe, C.A.J. *et al.* Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. *Clinical pharmacokinetics* **48**, 371-85 (2009).
16. Cella, M., Gorter de Vries, F., Burger, D., Danhof, M. & Della Pasqua, O. A model-based approach to dose selection in early pediatric development. *Clinical pharmacology and therapeutics* **87**, 294-302 (2010).
17. Anderson, B.J., van Lingen, R.A., Hansen, T.G., Lin, Y.-C. & Holford, N.H.G. Acetaminophen developmental pharmacokinetics in premature neonates and infants: a pooled population analysis. *Anesthesiology* **96**, 1336-45 (2002).
18. Cella, M., Zhao, W., Jacqz-Aigrain, E., Burger, D., Danhof, M. & Della Pasqua, O. Paediatric drug development: are population models predictive of pharmacokinetics across paediatric populations? *British journal of clinical pharmacology* **72**, 454-64 (2011).
19. Cella, M., Knibbe, C., de Wildt, S.N., Van Gerven, J., Danhof, M. & Della Pasqua, O. Scaling of pharmacokinetics across paediatric populations: the lack of interpolative power of allometric models. *British journal of clinical pharmacology* **74**, 525-35 (2012).
20. Bergshoeff, A. *et al.* Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
21. Musiime, V. *et al.* Pharmacokinetics and acceptability of once- versus twice-daily lamivudine and abacavir in HIV type-1-infected Ugandan children in the ARROW Trial. *Antiviral therapy* **15**, 1115-24 (2010).
22. Paediatric European Network for Treatment of AIDS (PENTA). Pharmacokinetic study of once-daily versus twice-daily abacavir and lamivudine in HIV type-1-infected children aged 3-<36 months. *Antiviral therapy* **15**, 297-305 (2010).
23. Mandema, J.W., Verotta, D. & Sheiner, L.B. Building population pharmacokinetic--pharmacodynamic models. I. Models for covariate effects. *Journal of pharmacokinetics and biopharmaceutics* **20**, 511-28 (1992).
24. Harnisch, L., Shepard, G.P. and Della Pasqua, O. Modeling and Simulation as a Tool to Bridge Efficacy and Safety Data in Special Populations. *CPT: Pharmacometrics & Systems Pharmacology* (2013).
25. Chan, P.L.S., Jacqmin, P., Lavielle, M., McFadyen, L. & Weatherley, B. The use of the SAEM algorithm in MONOLIX software for estimation of population pharmacokinetic-pharmacodynamic-viral dynamics parameters of maraviroc in asymptomatic HIV subjects. *Journal of pharmacokinetics and pharmacodynamics* **38**, 41-61 (2011).

SECTION III

Simplified dosing regimens
in children

A MODEL-BASED APPROACH FOR THE EVALUATION OF ONCE DAILY DOSING OF LAMIVUDINE IN HIV-INFECTED CHILDREN

Chiara Piana, Wei Zhao, Kim Adkison, David Burger , Evelyne Jacqz-Aigrain, Meindert Danhof , Oscar Della Pasqua

In press - Br J Clin Pharmacol

SUMMARY

Aim: Little attention has been paid to the effects of compliance and prescription practice on treatment outcome in HIV-infected children. In this context, an evaluation of the role of covariates on pharmacokinetics is required to establish the impact of differences in dosing regimen. Here we investigate whether a once daily dosing regimen of lamivudine provides comparable exposure to the currently approved paediatric regimen.

Methods: A hypothetical group of 180 patients between 3 months and 12 years old was used to evaluate the impact of body weight on systemic exposure to lamivudine. Simulation scenarios were evaluated using AUC and C_{max} as parameters of interest. The analysis was performed using a population pharmacokinetic model previously implemented in NONMEM v.6.2.

Results: The simulations show that once daily dosing of lamivudine yields comparable exposure to historical values observed in children and adults, both for liquid and solid dosage forms. Simulated steady-state AUC₀₋₂₄ and C_{max} values after once daily doses ranged respectively from 9.95 mg•h/L and 1.9 mg/L for children lighter than 14 kg to 13.75 mg•h/L and 3.0 mg/L for children heavier than 30 kg. These values are comparable or higher than historical values observed after once daily dosing in children and adults.

Conclusions: Our findings illustrate how dosing regimens can be evaluated taking into account the effects of developmental growth on drug disposition. Most importantly, they suggest that the reduction in dosing frequency to once daily warrants safe, efficacious exposure, while representing an improvement in treatment acceptability and adherence.

6.1. INTRODUCTION

Historically prescription practice and patient compliance have not been considered as factors determining the successful use of antiretroviral drugs in HIV-infected children. Increasing evidence now shows that not only the availability of suitable paediatric dosage forms, but also dosing frequency can be an important determinant of compliance and consequently of treatment outcome (1). In fact, a significant correlation between lower pill burden and better virological outcome has been observed for antiretroviral drugs (2). In addition, numerous surveys of HIV patients in both the United States and Europe indicate that there is a strong preference for once daily dosing and compact therapy (3) and according to several studies once daily regimens are significantly better adhered to than other dosing regimens (4). It is well known that current combination antiretroviral therapy (cART) regimens require large numbers of pills or capsules to be taken several times per day and the overall pill burden may thus be too large to permit adherence for periods of many years. Differences in dosing regimen and/or dosing frequency may clearly constitute a burden for patients and in particular for younger children.

The availability of fixed-dose combinations and the possibility to administer all the drugs as once daily regimen may be very advantageous, with direct implications for adherence to therapy and for the overall treatment outcome (5,6,7). The use of a simple once daily cART regimen may therefore be a powerful solution to optimise treatment adherence and patient's quality of life (8). Particularly in the paediatric field, there may be considerable benefits for both children and caregivers if dosing frequency can be reduced to once daily for all drugs in the regimen, allowing minimal interference with everyday school and home activities. Furthermore, one specific concern with older children is the stigma of taking medications during the day or having friends discover that they have an illness, so limiting the number of times a child has to take a medication can significantly improve not just compliance, but well-being.

Once daily dosing may provide the flexibility to maximise adherence according to individual circumstances – particularly in resource limited settings where most HIV-infected children live (9). For example, caregivers who are sellers in the market may find it hard to give drugs in the morning if they leave before the child is awake. Caregivers who work evenings may have to rely on others to administer evening doses. In addition, reducing administration frequency may significantly reduce medication error.

Lamivudine (3TC), a nucleoside reverse transcriptase inhibitor commonly administered in combination antiretroviral therapy to HIV-infected children (10), was initially administered twice daily in both adults and children. Lamivudine enters infected lymphocytes and is progressively phosphorylated by intracellular enzymes to the active moiety, lamivudine-5' triphosphate, which acts as a chain terminator. The active intracellular lamivudine-triphosphate has a long half-life (16-19 hours) relative to the half-life of parent lamivudine in plasma (5-7 hours) (11). The long half-life of lamivudine-triphosphate coupled with intracellular pooling of precursor

lamivudine-diphosphate supported the investigation of once daily dosing in adults. In fact, a once daily dose regimen was subsequently approved for adults based on clinical studies which showed equivalent antiviral activity (12) and equivalent area-under-the-curve [AUC_{0-24}] of plasma lamivudine and intracellular lamivudine-triphosphate (13) following once versus twice daily dosing of the same total daily dose. Even though a formal concentration-antiviral effect relationship is lacking due to the difficulties in routinely measuring the intracellular lamivudine-triphosphate concentrations, the plasma AUC_{0-24} of lamivudine can be considered the best plasma predictor of antiviral activity based on the mechanism of action and long half-life of the active moiety.

Currently lamivudine is labelled for twice daily administration in children based on clinical trials which demonstrated antiviral activity at doses yielding similar exposure to those observed in adults. Given the mechanism of action of lamivudine (14) the exposure-antiviral response to HIV infection is likely to be similar between children and adults. Therefore, a once daily dose regimen in children that can match the AUC_{0-24} of the approved twice daily regimen in children or the once or twice daily regimen in adults should demonstrate equivalent antiviral activity in children. To that purpose, several studies have been conducted to explore the pharmacokinetics and feasibility of once daily dosing in children (15, 16). Nevertheless, an integrated, model-based evaluation of the impact of developmental growth factors has not been performed for once daily dosing in children. It can be envisaged that the use of once daily dosing in children will allow alignment with the approval of once daily fixed-dose combination antiretroviral pills for the adult indication. The aim of this study is therefore to assess whether lamivudine pharmacokinetics after once daily dosing is comparable to lamivudine pharmacokinetics after twice daily administration to HIV-infected children between 3 months and 12 years old. The use of simulation scenarios is proposed as the basis for evidence synthesis on the suitability of this new regimen in children. Simulated pharmacokinetic profiles are characterised in a large hypothetical paediatric cohort to determine the dose rationale without the requirement for further enrolment of children into a clinical trial (17, 18).

The relevance of this type of model-based extrapolation exercise has been recently highlighted in the concept paper of the EMA (19). The document emphasises how extrapolations enable one *“to extend information and conclusions available from studies in one or more subgroups of the patient population (source population), or in related conditions or with related medicinal products, to make inferences for another subgroup of the population (target population), or condition or product, thus reducing the need to generate additional information (types of studies, design modifications, number of patients required) to reach conclusions for the target population, or condition or medicinal product”*.

6.2. METHODS

Simulations were performed to compare the systemic exposure of lamivudine after once daily dosing to historical values in children and adults and to explore how differences in demographic covariates affect steady-state exposure. The hypothetical population was represented by children between 3 months and 12 years old. For the purposes of our analysis, children were split into various age groups, each with 5 patients with different body weight (n=180). The correlation between age and body weight was based on the WHO weight-for-age tables (20). Lamivudine total daily doses were determined according to the currently recommended dose and method of administration, as indicated in the latest Summary of Product Characteristics (21).

A one-compartment model with first order absorption and elimination processes previously developed and validated by our group was used to simulate the pharmacokinetic profiles (Chapter 3). The model was built using pharmacokinetic data in 77 HIV-infected children receiving lamivudine both as twice and once daily dosing regimens. Body weight was found to be exponentially correlated to clearance and volume of distribution. Given that formulation was not found to influence the pharmacokinetic parameters, the same model was used to predict lamivudine pharmacokinetics in children receiving tablets or solution.

The frequency and times for pharmacokinetic sampling were based on a serial sampling scheme to mimic current practice with regard to estimating AUC over the dosing interval. Concentration vs. time data was then integrated using the trapezoidal rule to ensure realistic estimates of variability, as observed in a typical non-compartmental analysis. The hypothetical experimental protocol is depicted in figure 1. Given that a significant concentration-effect relationship for lamivudine could not be found in the past, the adequacy of the simulated dosing regimens was assessed graphically by determining the fraction of the paediatric population reaching systemic exposure comparable to AUC_{0-24} values previously observed in studies of adults on approved once and twice daily regimens and children on approved twice daily regimens. Cmax values of the paediatric population were also compared to historical values of Cmax from previous clinical trials. Simulations were performed using NONMEM version 6.2. Results were graphically summarised using R 2.8.2.

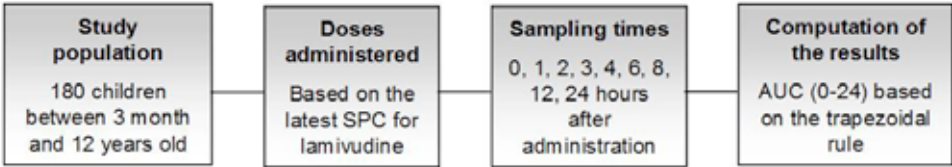


Figure 1 Diagram depicting the hypothetical experimental protocol

6.3. RESULTS

Simulations were performed using a population pharmacokinetic model previously developed and validated by our group. The goodness of fit and visual predictive checks are shown in figure 2.

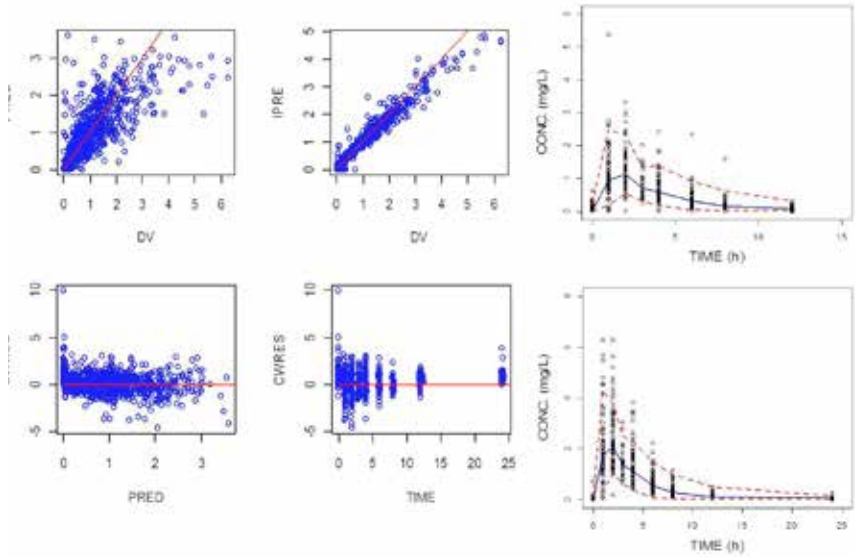


Figure 2 (a) Goodness-of-fit. Left upper panel shows population prediction (PRED) vs. observed concentration values (DV). Right upper panel shows individual predictions (IPRED) vs. observed concentration values (DV). Left lower panel shows conditional weighted residuals (CWRES) vs. population predictions (PRED). Right lower panel shows conditional weighted residuals (CWRES) vs. time (TIME). (b) Visual predictive check (VPC) of the population pharmacokinetic model for lamivudine.

Based on the original parameter estimates, the distribution of the area under the curve (AUC_{0-24}) and peak concentration (Cmax) values associated with a once daily dosing regimen for lamivudine were evaluated in a hypothetical group of paediatric patients. In total, the simulated population consisted of 180 patients between 3 months and 12 years old, which represent a population with comparable demographic characteristics of HIV-infected children in a typical clinical setting. The demographic characteristics of the simulated population is summarised in Table 1. In table 2 the doses of lamivudine administered to the simulated population are depicted.

Table 1 Demographic characteristics of the simulated paediatric population

	Overall	<14 kg	14-21 kg	21-30 kg	>30 kg
SUBJECTS	180	85	34	31	30
MEDIAN AGE (years)	3.5	0.91	4.5	8	10.5
MIN (years)	0.25	0.25	2	5	7.5
MAX (years)	12	3.5	7.5	11	12
MEDIAN WEIGHT (kg)	14.9	9.73	17.2	24.9	35.9
MIN (kg)	5.41	5.41	14.1	21.1	30.2
MAX (kg)	53.9	13.6	20.7	29.1	53.9

The simulation results are presented graphically in figures 3 and 4, which show the comparison between the simulated distributions of the secondary pharmacokinetic parameters [AUC₀₋₂₄ and Cmax] and historical data from previous clinical trials with lamivudine in children and adults (22, 23). For completeness, the pharmacokinetic parameters of lamivudine are presented in table 3. Box plots show that the predicted lamivudine exposure reached after once daily dosing was comparable or higher in every weight range than the exposure reached in historical trials where lamivudine was administered at approved once or twice daily doses to adults and twice daily doses to children. The predicted Cmax values on the once daily regimen exceeded those of the twice daily regimen in children; however, there was considerably overlap of the predicted Cmax values with those observed in adult subjects on the once daily regimen.

Table 2 Currently recommended doses of lamivudine in children

Weight Band	Lamivudine Dose Regimen	Lamivudine Total Daily Dose
<14kg	Oral solution (4mg/kg) twice daily	8mg/kg/day
14 to 21kg	One-half tablet (75mg) twice daily	150mg
>21 to <30kg	One-half tablet (75mg) in the morning; One whole tablet (150mg) in the evening	225mg
30kg	One whole tablet (150mg) twice daily	300mg

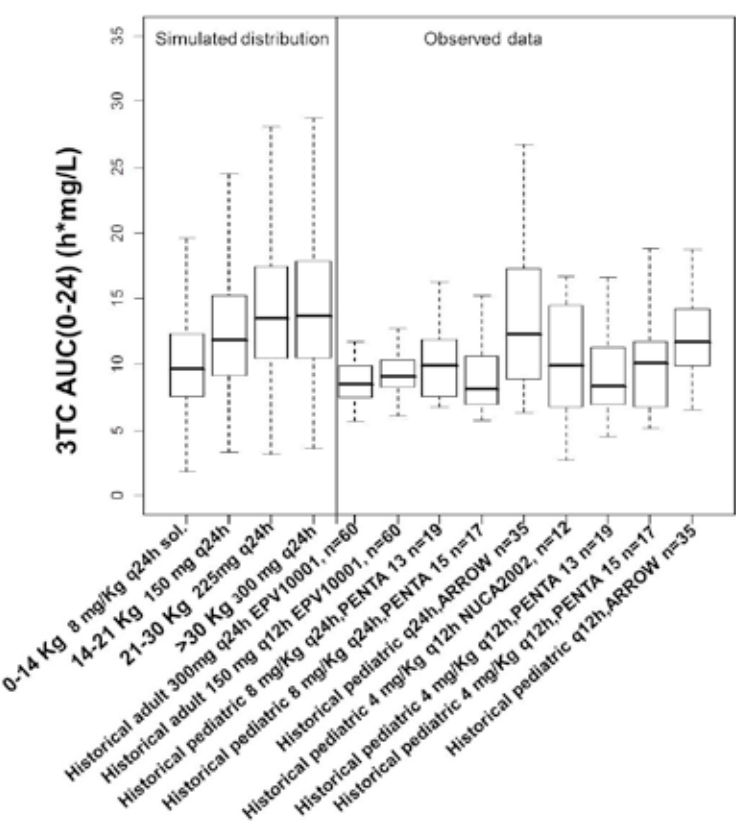


Figure 3 Box plots showing the comparison between simulated distributions of lamivudine AUC₀₋₂₄ after once daily dosing and historical data from clinical trials. Box represents median, 25th and 75th percentiles, bars represent 10th and 90th percentiles. Simulated distributions (N= 500 replicate trials) are comparable or higher than historical data in each weight range.

6.4. DISCUSSIONS AND CONCLUSIONS

Evidence synthesis by modelling and simulation

Undoubtedly, the use of once daily dosing of antiretroviral drugs in HIV-infected children may offer significant clinical advantages, especially in resource limited countries. There can be several benefits for both children and caregivers and adherence may be strongly maximised, with consequent improvements in treatment outcome.

Given that previous studies have shown similar pharmacokinetics between once and twice daily dosing and evidence on the preference of caregivers for once daily regimen, evidence synthesis rather than new evidence generation needs to be considered to support once daily dosing of lamivudine in HIV-infected children. We have used a model-based approach to evaluate whether differences exist in the pharmacokinetics of lamivudine after once daily dosing, as comparable to the achieved exposure after twice daily administration. Our results clearly show that simulation scenarios offer the possibility to evaluate the potential implications of changes in dosing regimen based on existing evidence in the adult population and limited experience in children.

It is unfortunate that historically population pharmacokinetic models have been used primarily as an alternative estimation method, with simulations being performed primarily as a diagnostic procedure during model validation, rather than as an evidence synthesis tool for subsequent decision making. Yet, evidence synthesis and in particular extrapolations are often used implicitly in many situations involving clinical or regulatory decisions, e.g. when extending conclusions from trial populations to the general populations. By applying a model-based approach, one can ensure explicit and systematic assessment of the assumptions, clinical implications and risks associated with the use of extrapolations.

Once daily dosing regimen: systemic exposure

Simulation scenarios show that lamivudine AUC_{0-24} reached after once daily dosing are comparable to historical values in children on a twice daily regimen of lamivudine and adults receiving the approved once or twice daily lamivudine regimens. Figure 3 shows that the youngest group of children (between 0 and 14 kg) had quite a lower exposure compared to the older, heavier children. This fact, previously shown by Burger *et al* (24), could be partly explained by a slightly lower dose that the small children receive (as shown in table 3). Effectively, higher mg/kg doses are administered with the score tablet dosage regimens due to the pre-defined tablets strengths (either 75mg half tablet or 150mg whole tablet) and because of the weight band cut-offs selected to minimise under-dosing in heavier children. Therefore, lighter children in the same weight band receive doses that are substantially higher than the 8mg/kg/day when the solution is administered. There may also be some effect of the formulation, since these children receive solution and the heavy ones receive tablets, as shown in a recent study from Kasirye *et al.* (25)

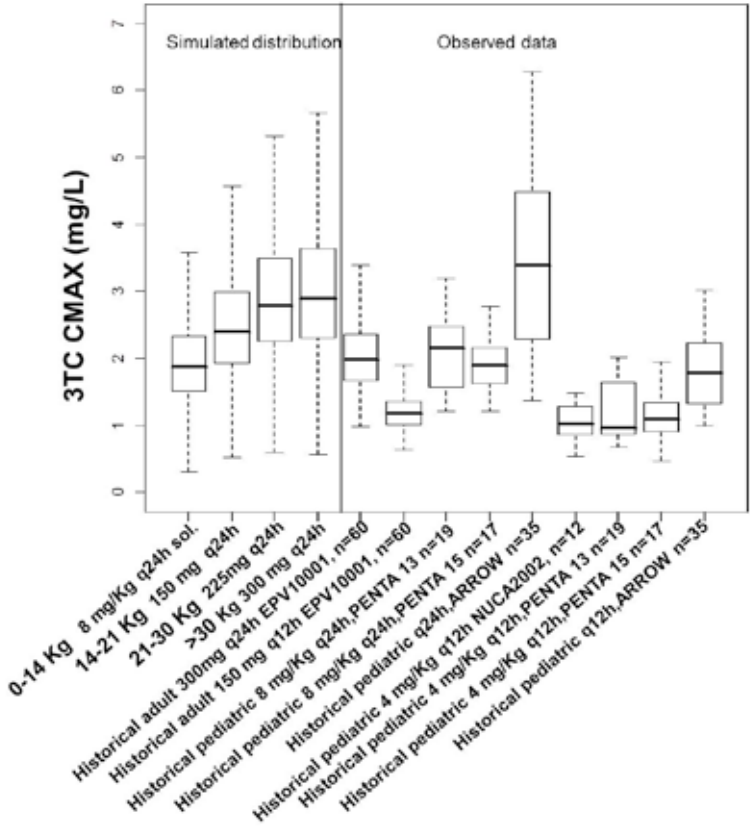


Figure 4 Box plots showing the comparison between simulated distributions of lamivudine Cmax after once daily dosing and historical data from clinical trials. Box represents median, 25th and 75th percentiles, bars represent 10th and 90th percentiles. Simulated distributions (N= 500 replicate trials) are comparable or higher than historical data in each weight range

Table 3. Summary of the pharmacokinetic parameters of the simulated population. Values are presented as geometric mean (95% CI) except for dose which is presented as median (range)

	OVERALL	< 14 kg	14-21 kg	21-30 kg	> 30 kg
Dose (mg/kg)	8.51 (5.55-10.66)	8.45 (7.84-9.25)	8.72 (7.21-10.61)	9.01 (7.75-10.66)	8.34 (5.55-9.91)
AUC ₀₋₂₄ (mg•h/L)	11.26 (10.70-11.86)	9.65 (9.01-10.36)	11.83 (10.51-13.26)	13.47 (11.84-15.37)	13.68 (12.01-15.52)
Cmax (mg/L)	2.25 (2.14-2.362)	1.87 (1.75-2.01)	2.38 (2.13-2.66)	2.79 (2.50-3.14)	2.87 (2.53-3.22)
CL/F (L/h)	15.19 (14.42-16.04)	10.65 (9.89-11.5)	16.14 (14.39-18.28)	21.07 (18.58-23.51)	27.68 (24.26-31.69)
CL/F/ (L/h/kg)	0.96 (0.91-1.020)	1.12 (1.04-1.211)	0.93 (0.83-1.060)	0.84 (0.74-0.950)	0.74 (0.65-0.850)

However, such effect is probably not large enough, as it could not be identified as a significant covariate during model building. Lastly, it is worth mentioning that distributional mechanisms may also be implicated in low exposure in younger children (26, 27). Although simulated exposures varied across the paediatric weight range, children in all weight bands showed predicted lamivudine AUC₀₋₂₄ values after once daily administration comparable to or higher than the reference values previously observed in children on twice daily regimen or adults on once daily or twice daily dosing regimen. Given a similar exposure-antiviral response relationship for HIV infection in adults and children, a once daily regimen that matches the AUC₀₋₂₄ of the approved twice daily regimen in children and the once daily regimen in adults should demonstrate equivalent antiviral activity in children.

Once daily dosing regimen: peak concentrations

As anticipated from drugs showing linear pharmacokinetics, the reduction in dosing frequency resulted in an increase in median Cmax by approximately two-fold. Figure 4 shows that the maximum peak concentration reached after once daily dosing is much higher compared to twice daily administration. Once daily administration of lamivudine to HIV-infected children also results in higher Cmax than the historical values observed in adults and children during previous clinical trials in which twice daily dosing has been used. Given that once daily lamivudine was approved for use in adults based on good safety and efficacy and the positive tolerability and safety profile of once daily lamivudine was observed in small studies of children (28), the predicted

increase in Cmax after once daily administration is unlikely to result in a higher risk of adverse events. Again Cmax values appear to be slightly lower for children lighter than 14 kg. However the simulated maximum peak concentration in this group of children is comparable with reference Cmax values in adults receiving once daily dosing and higher than reference Cmax values in children receiving lamivudine twice daily and in adults receiving lamivudine twice daily.

Clearly one of the major concerns about once daily administration of antiretroviral drugs is the higher risk of viral failure. In adults it has been shown that once daily lamivudine in combination with zidovudine and efavirenz would provide comparable treatment outcome as twice daily lamivudine (12). It has also been shown that didanosine, another NRTI with similar pharmacokinetic properties as lamivudine, allows for once daily administration without increased risk of viral failure (29–31). Regarding the increased risk of drug resistance, previous studies demonstrated that once daily dosing of antiretrovirals is strongly correlated to increased patient adherence to therapy. Given that high levels of adherence may avoid development of resistance, the use of once daily dosing regimen is not expected to increase the probability of virus mutations and drug resistance.

Limitations

One of the main limitations in our study was that lamivudine plasma pharmacokinetics can only be considered as a limited marker of drug exposure as it is the intracellular lamivudine triphosphate metabolite that becomes pharmacologically active. However, no alternative is available due to the requirements for adequate sampling of intracellular concentrations of nucleoside transcriptase inhibitor triphosphate, which is logistically and technically difficult. This is further complicated by the volume of blood required for the bioanalysis of intracellular lamivudine triphosphate concentrations, which makes serial evaluations impractical for paediatric patients. Instead, we have made explicit assumptions about the use of plasma concentrations, namely that equilibrium between plasma and intracellular concentrations is rapidly reached and drug distribution into cells is driven by a first order process, without the risk of saturation occurring within the range of concentrations observed after once or twice daily dosing.

Conclusions

In conclusion, the possibility of evaluating the implication of different dosing regimens using a model-based approach shows one of the various applications of virtual clinical trials in paediatric clinical pharmacology research. Our findings strongly suggest that when the same total daily lamivudine dose is administered, the reduction in dosing frequency to once daily does not represent a potential risk of under- or over-dosing in the paediatric population. Taking into account the evidence regarding acceptability and adherence in previous paediatric and adult HIV trials, the current results provides evidence for an alternative, once daily dosing regimen, with the advantages of improved adherence and consequently efficacy and clinical outcome for children, particularly in resource limited settings.

References

1. Haberer, J. & Mellins, C. Pediatric adherence to HIV antiretroviral therapy. *Current HIV/AIDS reports* **6**, 194-200 (2009).
2. Bartlett, J.A., DeMasi, R., Quinn, J., Moxham, C. & Rousseau, F. Overview of the effectiveness of triple combination therapy in antiretroviral-naïve HIV-1 infected adults. *AIDS (London, England)* **15**, 1369-77 (2001).
3. Moyle, G. Once-daily therapy: less is more. *International journal of STD & AIDS* **14 Suppl 1**, 1-5 (2003).
4. Claxton, A.J., Cramer, J. & Pierce, C. A systematic review of the associations between dose regimens and medication compliance. *Clinical therapeutics* **23**, 1296-310 (2001).
5. Manfredi, R. & Calza, L. Recent availability of two novel, fixed formulations of antiretroviral nucleoside analogues: a 12-month prospective, open-label survey of their practical use and therapeutic perspectives in antiretroviral-naïve and -experienced patients. *AIDS patient care and STDs* **22**, 279-90 (2008).
6. Chokephaibulkit, K. *et al.* Pharmacokinetics and safety of a new paediatric fixed-dose combination of zidovudine/lamivudine/nevirapine in HIV-infected children. *Antiviral therapy* **16**, 1287-95 (2011).
7. Rosso, R. *et al.* Patient-reported outcomes and low-level residual HIV-RNA in adolescents perinatally infected with HIV-1 after switching to one-pill fixed-dose regimen. *AIDS care* **24**, 54-8 (2012).
8. Taburet, A.-M., Paci-Bonaventure, S., Peytavin, G. & Molina, J.-M. Once-daily administration of antiretrovirals: pharmacokinetics of emerging therapies. *Clinical pharmacokinetics* **42**, 1179-91 (2003).
9. Portsmouth, S.D., Osorio, J., McCormick, K., Gazzard, B.G. & Moyle, G.J. Better maintained adherence on switching from twice-daily to once-daily therapy for HIV: a 24-week randomized trial of treatment simplification using stavudine prolonged-release capsules. *HIV medicine* **6**, 185-90 (2005).
10. Achenbach, C.J., Scarsi, K.K. & Murphy, R.L. Abacavir/lamivudine fixed-dose combination antiretroviral therapy for the treatment of HIV. *Advances in therapy* **27**, 1-16 (2010).
11. Moore, K.H. *et al.* The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. *AIDS (London, England)* **13**, 2239-50 (1999).
12. DeJesus, E. *et al.* Once-daily versus twice-daily lamivudine, in combination with zidovudine and efavirenz, for the treatment of antiretroviral-naïve adults with HIV infection: a randomized equivalence trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **39**, 411-8 (2004).
13. Yuen, G.J. *et al.* Equivalent steady-state pharmacokinetics of lamivudine in plasma and lamivudine triphosphate within cells following administration of lamivudine at 300 milligrams once daily and 150 milligrams twice daily. *Antimicrobial agents and chemotherapy* **48**, 176-82 (2004).
14. Perry, C.M. & Faulds, D. Lamivudine. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV infection. *Drugs* **53**, 657-80 (1997).
15. Bergshoeff, A. *et al.* Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
16. Paediatric European Network for Treatment of AIDS (PENTA). Pharmacokinetic study of once-daily versus twice-daily abacavir and lamivudine in HIV type-1-infected children aged 3-<36 months. *Antiviral therapy* **15**, 297-305 (2010).
17. Bellanti, F. & Della Pasqua, O. Modelling and simulation as research tools in paediatric drug development. *European journal of clinical pharmacology* **67 Suppl 1**, 75-86 (2011).
18. De Cock, R.F.W., Piana, C., Krekels, E.H.J., Danhof, M., Allegaert, K. & Knibbe, C.A.J. The role of population PK-PD modelling in paediatric clinical research. *European journal of clinical pharmacology* **67 Suppl 1**, 5-16 (2011).
19. European medicines Agency. Concept paper on extrapolation of efficacy and safety in medicine development (2013). Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/04/WC500142358.pdf (last accessed 20 May 2013)
20. The WHO Child Growth Standards. Available at: <http://www.who.int/childgrowth/en/> (last accessed 20 May 2013)
21. Summary of Product Characteristics of lamivudine. Available at: <http://apps.who.int/prequal/whopar/whoparproducts/HA414Part4v1.pdf> (last accessed 20 May 2013)

22. GlaxoSmithKline Document Number UCR/95/004. Study ID NUCA2002. A phase I/II study of 3TC (GR109714X) in children with HIV infection.
23. GlaxoSmithKline Document Number RM2000/00258/00. Study ID EPV10001. An Open-Label, Randomized, Two-way Crossover Study to Compare the Steady-State Pharmacokinetics of Lamivudine and Lamivudine Triphosphate Following Lamivudine 300mg Once Daily Versus EPIVI.
24. Burger, D.M. *et al.* Age-dependent pharmacokinetics of lamivudine in HIV-infected children. *Clinical pharmacology and therapeutics* **81**, 517-20 (2007).
25. Kasirye, P. *et al.* Pharmacokinetics of antiretroviral drug varies with formulation in the target population of children with HIV-1. *Clinical pharmacology and therapeutics* **91**, 272-80 (2012).
26. Minuesa, G. *et al.* Transport of lamivudine [(-)-beta-L-2',3'-dideoxy-3'-thiacytidine] and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2, and 3. *The Journal of pharmacology and experimental therapeutics* **329**, 252-61 (2009).
27. Anderson, P.L., Lamba, J., Aquilante, C.L., Schuetz, E. & Fletcher, C.V. Pharmacogenetic characteristics of indinavir, zidovudine, and lamivudine therapy in HIV-infected adults: a pilot study. *Journal of acquired immune deficiency syndromes (1999)* **42**, 441-9 (2006).
28. LePrevost, M. *et al.* Adherence and acceptability of once daily Lamivudine and abacavir in human immunodeficiency virus type-1 infected children. *The Pediatric infectious disease journal* **25**, 533-7 (2006).
29. Roca, B., Lapuebla, C. & Vidal-Tegedor, B. HAART with didanosine once versus twice daily: adherence and efficacy. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* **9**, 195-200 (2005).
30. Kazatchkine, M.D. *et al.* Didanosine dosed once daily is equivalent to twice daily dosing for patients on double or triple combination antiretroviral therapy. The AI454-147 Team. *Journal of acquired immune deficiency syndromes (1999)* **24**, 418-24 (2000).
31. Monno, L. *et al.* Comparison of once and twice daily dosing of didanosine in combination with stavudine for the treatment of HIV-1 infection. AI 454-146 Team. *Antiviral therapy* **4**, 195-202 (1999).

SUITABILITY OF ONCE DAILY DOSING ABACAVIR IN CHILDREN

Chiara Piana, Wei Zhao, Kim Adkison, David Burger,
Evelyne Jacqz-Aigrain, Oscar Della Pasqua

Submitted for publication

SUMMARY

This investigation is aimed at evaluating whether abacavir exposure reached after once daily dosing regimen is comparable to abacavir exposure following the currently recommended twice daily regimen in HIV-infected children between 3 months and 12 years old. Simulation scenarios were carried out using a pharmacokinetic model previously developed in HIV-infected children. Abacavir exposure was simulated in a hypothetical paediatric population (n=180) between 3 months and 12 years old. Simulations were performed in NONMEM v6 and R 2.8.1 was used to graphically summarise the results. According to the evaluated simulation scenarios, abacavir exposure $[AUC_{0-24}]$ reached after once daily dosing in children is comparable to previous values observed in children on twice daily dosing regimens and in adults on once or twice daily dosing. Based on our findings, the reduction in abacavir dosing frequency from twice daily to once daily in HIV-infected children provides appropriate values of exposure and may represent an opportunity for the improvement of adherence and clinical outcome.

7.1. INTRODUCTION

Poor adherence is among the main causes of reduced activity of antiretroviral drugs in children (1). Failure to take the prescribed doses of antiretroviral drugs may lead to ongoing viral replication in the presence of drug and the selection of drug-resistant HIV strains (2, 3). Adherence has been shown to be a strong predictor of therapeutic impact in HIV medication in several studies (4, 5). Wiener *et al.* reported that among children with an HIV-1 RNA viral load < 10000, 75% had taken 100% of their medication doses in the previous week, while among those with a viral load

of >100000, only 36% reported taking all of their medication (6). Pediatric adherence mainly depends on a caregiver to administer the medication. In limited resource countries, where mostly HIV-infected children live, it is sometimes problematic to identify someone who can be responsible for drug administration, especially because this person may be impaired by complications of HIV infection or may need to work for most of the day. Once daily antiretroviral therapy is one possible approach to improve treatment outcome, as it simplifies the caregiver's role and presumably increases paediatric adherence. An ideal once daily drug should have a favorable pharmacokinetic profile such that standard dosing would provide a high probability of efficacy and a low probability of toxicity (7). Maintaining drug plasma concentrations above a certain threshold is generally believed to be related to the efficacy of antiretroviral agents (8). Therefore reliable bioavailability and a long half-life for the active moiety are the most important characteristics for once daily antiretroviral agents.

Abacavir (ABC) is a nucleoside reverse transcriptase inhibitor (NRTI) extensively prescribed in HIV paediatric combination therapy (9–13). Abacavir tablets are 83% bioavailable after oral administration and rapidly reach peak plasma concentrations between 0.63 and 2.5 hours (14). Abacavir is phosphorylated to its intracellular anabolite, carbovir triphosphate (CVB-TP), which inhibits transcription of HIV viral RNA to DNA by competing with an endogenous nucleotide transcriptase enzyme (15). Since the pharmacologically active moiety of abacavir is the intracellular CBV-TP, its pharmacokinetic profile is essential for determining the appropriate dosing interval for abacavir. Although CBV-TP pharmacokinetic parameters are difficult to estimate due to large within-subject variability, several studies demonstrated a half-life for CBV-TP greater than 12 hours, supporting once daily abacavir administration (16–18).

Abacavir is currently administered as once daily regimen to adults and twice daily to children. The possibility to administer abacavir according to a once daily dosing regimen in children has been demonstrated in several studies. PENTA 15 study showed comparable plasma abacavir exposure in terms of AUC_{0-24} for twice and once daily dosing in children between 3 months and 3 years old administered the oral solution formulation (19); PENTA 13 study and the sub-study of the ARROW trial showed similar twice daily and once daily pharmacokinetics in children between 2 and 13 years old receiving the oral solution or tablet formulations (20, 21). These studies also showed that once daily dosing was preferred to twice daily dosing by children and their caregivers. Further investigation is however needed to assess the feasibility of once daily abacavir in a large group of children.

The aim of this study is to evaluate, using modelling and simulation tools, whether abacavir exposure after once daily is comparable to abacavir exposure following twice daily regimens in HIV infected children between 3 months and 12 years old. The use of a previously developed pharmacokinetic model combined with simulation scenarios allows performing the investigation in a large hypothetical population of HIV-infected children.

7.2.METHODS

Clinical data

Historical clinical data of abacavir were used for comparison with the data from the simulated population of children following once daily dosing regimen. Several historical studies in which abacavir was administered to children (ACTG330 (twice daily) (22), CNAA1001 (twice daily) (23), PENTA 13 (twice daily and once daily) (20), PENTA 15 (twice daily and once daily) (19), ARROW-part 1 (twice daily and once daily) (21)) and to adults (CNAA2001 (twice daily) (24), CAL10001 (once daily) (25), CAL102120 (once daily) (26)) were used during the investigation. In table 1 an overview of each trial is provided in terms of study design, objective and number of subjects. The studies have been conducted in full conformance with the principles of the Declaration of Helsinki and with the local laws and regulations concerning clinical trials. The protocols and the informed consent documents for each study have been formally approved by the relevant research ethics committee of each clinical site and by a national ethics body.

Pharmacokinetic model

A pharmacokinetic two-compartment model previously developed by our group (27) to describe abacavir disposition in HIV-children between 3 months and 12 years old has been used to simulate abacavir exposure in a hypothetical paediatric population. The model used for simulation purposes was a two-compartment model with first order absorption and elimination and body weight as a significant covariate for apparent clearance and volume of distribution of the central compartment.

Simulation scenarios- *in silico* trial protocol

Abacavir exposure following once daily administration was simulated in a hypothetical population of 180 children between 3 months and 12 years old (table 2). Each age group was represented by 5 children with different body weight. WHO weight-for-age tables (28) were used to correlate age and body weight in the simulated patients in order to guarantee a realistic age-body weight correlation. Abacavir was administered according to the currently recommended dose and method of administration (table 3), as indicated in the latest Summary of Product Characteristics (29).

The variable of interest were the area under the curve (AUC) and the peak concentration (C_{max}) associated with once daily abacavir administration based on the currently recommended doses in children. Abacavir formulation was not identified as a significant covariate; therefore the same model was used to simulate the administration of solution (for children with body weight lower than 14 kg) and tablets. The frequency and times for pharmacokinetic sampling were based on a serial sampling scheme for the purposes of estimating AUC over the dosing interval. Figure 1 depicts the hypothetical experimental setting. Integration of the concentration

time data was applied according to the trapezoidal rule to ensure realistic estimates of variability. 500 replicates were performed.

Simulations were performed using NONMEM version 6.2. R version 2.8.2 was used to graphically summarise the results.

Comparison with historical data

The simulated values of AUC and Cmax were compared with historical values from previous clinical trials in which abacavir was administered to children and to adults in order to assess whether abacavir once daily administration provides appropriate values of plasma exposure. Weighted mean and standard deviation of the historical trials were used for comparison. AUC mean and standard deviation in each body weight group were each compared with the historical values using a two-tailed Z-test and a p-value of 0.05. Non-normality in the data was addressed by using a log10-transformation. Given that higher values of Cmax were expected, no formal statistical comparison was deemed necessary for Cmax.

Table 1 Historical studies of abacavir in adults and children used for comparison

Study	Design	Objective	Subjects
CAL 102120	Non-randomised, open-label, two-period, pharmacokinetic study.	To assess the pharmacokinetics of intracellular CBV-TP at steady state following administration of 600 mg once daily and 300 mg twice daily ABC-containing regimens	30 HIV-infected adult subjects
ACTG 330	Open-label, dose-escalating study	To evaluate the pharmacokinetic features, safety, and tolerance of abacavir, given alone and in combination with other nucleoside antiretroviral agents, in symptomatic HIV-infected children.	44 HIV-infected children
CNA 2001	Open-label, parallel dosing cohorts	To determine the multiple-dose pharmacokinetics and pharmacodynamics of abacavir in HIV-1-infected subjects following oral administration of daily doses that ranged from 600 to 1,800 mg, with and without zidovudine	20 HIV-infected adults

PENTA 13	One arm, open label cross-over study	To compare plasma pharmacokinetics of twice daily versus once daily lamivudine and abacavir in children aged 2-13 years	14 HIV-infected children
PENTA 15	Cross-over, open-label pharmacokinetic multi-centre study	To compare plasma pharmacokinetic parameters of once versus twice daily dosing of abacavir in HIV-1 infected infants and children aged 3 months to <36 months.	20 HIV-infected children
ARROW part1	Randomized controlled study	To compare the pharmacokinetics of once versus twice-daily lamivudine and abacavir treatment using the World Health Organization recommended weight band dosing of scored adult tablets.	36 HIV-infected children
CNA 1001	Single-dose, open label study	To assess the safety of single oral doses of abacavir in HIV-infected children ≤13 years of age, to determine the pharmacokinetics of abacavir in HIV-infected infants and children, and to determine the effect of development age on the pharmacokinetics and metabolism of abacavir.	19 HIV-infected children
CAL 10001	Single-center, open-label, randomized, repeat single-dose, 3-way crossover study	To demonstrate the bioequivalence between a single tablet composed of 600mg abacavir and 300mg lamivudine versus the treatment of abacavir 2 x 300mg tablet and lamivudine 2 x 150mg tablet swallowed sequentially.	25 healthy adults

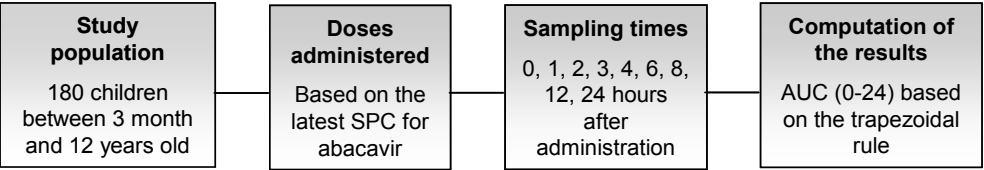


Figure 1 Diagram depicting the hypothetical experimental setting.

Demographic characteristics of the simulated paediatric population

	Overall	< 14 kg	14-21 kg	21-30 kg	> 30 kg
Subjects	180	85	34	31	30
Median Age (years)	3.5	0.91	4.5	8	10.5
Min (years)	0.25	0.25	2	5	7.5
Max (years)	12	3.5	7.5	11	12
Median weight(kg)	14.9	9.73	17.2	24.9	35.9
Min (kg)	5.41	5.41	14.1	21.1	30.2
Max (kg)	53.9	13.6	20.7	29.1	53.9

Table 3 Demographic characteristics of the simulated paediatric population

Weight Band	Abacavir Dose Regimen	Abacavir Total Daily Dose
<14kg	Oral solution (8mg/kg) twice daily	16mg/kg/day
14 to 21kg	One-half tablet (150mg) twice daily	300mg
>21 to <30kg	One-half tablet (150mg) in the morning; One whole tablet (300mg) in the evening	450mg
30kg	One whole tablet (300mg) twice daily	600mg

7.3. RESULTS

A hypothetical population of 180 children between 3 months and 12 years old was used during this investigation. The characteristics of the simulated paediatric population are summarised in table 2. Abacavir was administered once daily to the hypothetical population at the same total daily dose used in the currently approved twice daily regimens.

Abacavir area under the curve (AUC_{0-24}) and peak concentration (C_{max}) distributions following once daily administration were simulated in the abovementioned paediatric population based on a pharmacokinetic model previously developed by our group (27). The model has been selected based on graphical and statistical criteria and has been validated using several tools, namely bootstrap, mirror plots, NPDE and visual predictive check to guarantee its predictive power.

The simulation results are summarised graphically in figures 2 and 3, which show the comparison between the simulated distributions of the secondary pharmacokinetic parameters [AUC_{0-24} and C_{max}] and historical data from previous clinical trials with abacavir administered to children and to adults. Box plots show that the predicted abacavir exposure reached after once daily dosing of abacavir was comparable in every weight range to the exposure reached in historical trials where abacavir was administered at approved once or twice daily doses to adults and twice daily doses to children. A statistical test was performed to compare simulated AUC values with historical data. Based on a two-tailed Z-test the difference between the AUC values in each of the four groups and the historical data was not significant (p-value of 0.779, 0.1096, 0.09 and 0.1336, respectively).

Abacavir pharmacokinetic parameters of the simulated population are summarised in table 4.

Table 4 Summary of the pharmacokinetic parameters of the simulated population. Values presented as geometric mean (95% CI) except for dose which is presented as median (range)

	Overall	< 14 kg	14-21 kg	21-30 kg	> 30 kg
Dose (mg/kg)	16.51 (11.11-21.33)	16.39 (15.78-17.31)	17.45 (14.43-21.23)	18.03 (15.51-21.33)	16.68 (11.11-19.83)
AUC ₀₋₂₄ (mg•h/l)	14.1 (13.24-14.85)	12.39 (11.38-13.57)	14.79 (12.97-16.73)	16.35 (14.19-18.85)	15.87 (13.69-18.25)
Cmax (mg/L)	5.61 (5.29-5.95)	4.79 (4.42-5.24)	5.97 (5.21-6.87)	6.74 (5.81-7.85)	6.67 (5.76-7.71)
CL/F (L/h)	18.45 (17.61-19.28)	12.29 (11.52-13.08)	19.69 (17.87-21.58)	26.67 (24.08-29.93)	36.53 (33.06-40.61)
CL/F/kg (L/h/kg)	1.16 (1.11-1.22)	1.29 (1.21-1.37)	1.14 (1.04-1.25)	1.06 (0.96-1.19)	0.98 (0.89-1.09)

7.4. DISCUSSIONS AND CONCLUSIONS

Clinical implication of a once daily dosing regimen for abacavir

This investigation is aimed at assessing whether once daily administration of abacavir to HIV-infected children between 3 months and 12 years old provides appropriate values of exposure compared to historical trials in adults and children. Given that paediatric adherence is a major problem in antiretroviral combination therapy, abacavir once daily dosing to children may constitute a great advantage in improving patient compliance. It is well known that young children must rely on caregivers for drug administration. In limited resource countries, where caregivers may be unable to administer the medication for various reasons, compliance is a serious issue. The relation between poor adherence and drug failure has been clearly demonstrated in several studies. Therefore the possibility to reduce dosing frequency may be an effective solution to increase patient adherence and to improve treatment outcome.

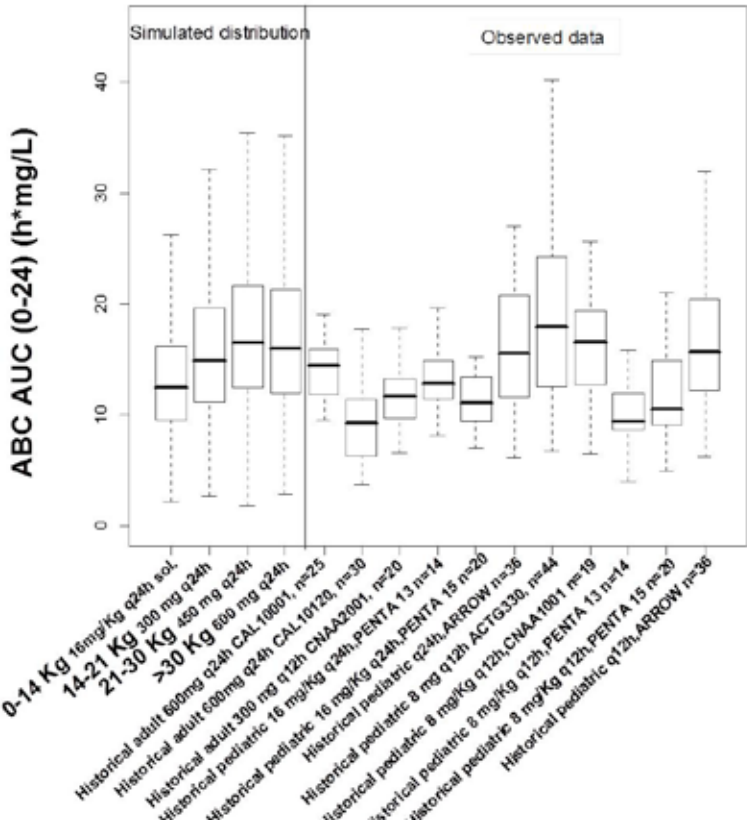


Figure 2 Box plots showing the comparison between simulated distributions of abacavir AUC₀₋₂₄ after once daily dosing and historical data from clinical trials. Box represents median, 25th and 75th percentiles, bars represent 10th and 90th percentiles. Simulated distributions (N= 500 replicate trials) are comparable or higher than historical data in each weight range.

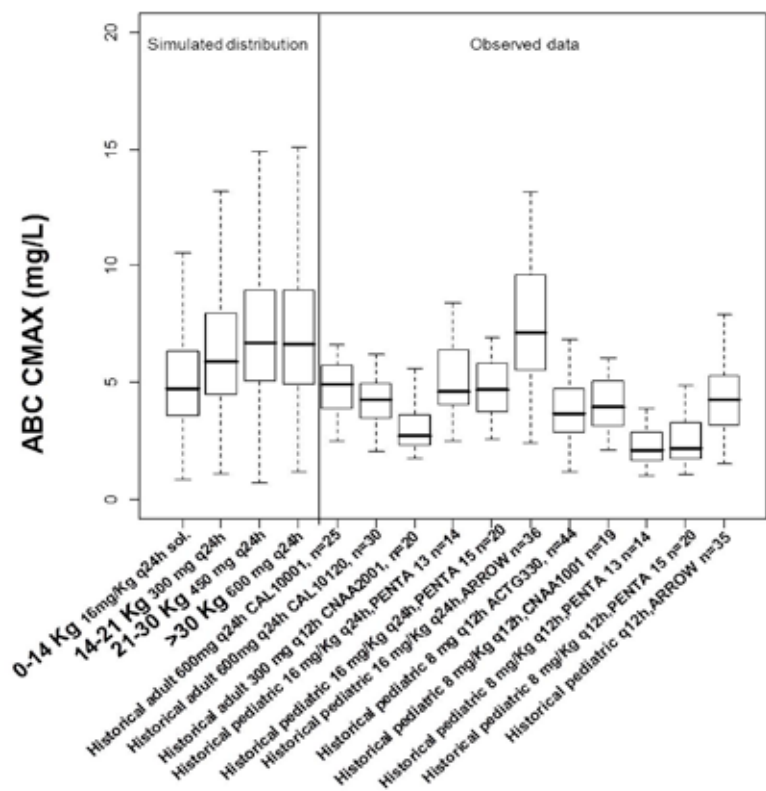


Figure 3 Box plots showing the comparison between simulated distributions of abacavir Cmax after once daily dosing and historical data from clinical trials. Box represents median, 25th, and 75th percentiles, bars represent 10th and 90th percentiles. Simulated distributions (N= 500 replicate trials) are comparable or higher than historical data in each weight range.

A model-based approach for the evaluation of alternative dosing regimens

In this investigation we use a model-based approach to simulate abacavir exposure in a large group of hypothetical HIV-infected children. The great advantage of a modelling and simulation approach in paediatrics is the possibility of exploring relevant scenarios without putting children into a clinical protocol (30). Simulations allow evaluation of a dosing regimen in a large population with a wide range of demographic characteristics which cannot be tested in reality for obvious ethical and technical issues. In this case we used a hypothetical population of 180 HIV-infected children uniformly distributed between 3 months and 12 years old and a previ-

ously validated pharmacokinetic model for simulation purposes. As shown in figures 2 and 3, simulated abacavir AUC₀₋₂₄ appears to be comparable to all the historical values deriving from clinical trials in children and adults based on twice daily or once daily dosing regimen except the historical AUC₀₋₂₄ value deriving from the ACTG330 trial, performed in children receiving abacavir as twice a day dosing regimen (figure 3). However, as shown in figure 2, the mean value of AUC₀₋₂₄ from the ACTG330 study is considerably higher compared to every other previous study. Moreover, a statistical comparison in which simulated AUC₀₋₂₄ values are compared with weighted mean and standard deviation of the historical values showed that the difference between the AUC₀₋₂₄ values in each weight group and the historical data is not statistically significant.

As shown in a similar analysis performed on lamivudine by our group (Chapter 6), the older children (>14 kg) have a higher exposure compared to younger children (<14 kg). This difference may be partly explained by a slightly higher mg/kg dose that the older children (>14 kg) are administered when given the tablet formulation (as shown in table 3), as for lamivudine. There may also be an effect of the formulation, since younger children (<14 kg) were assigned to receive solution and the older children (>14 kg) were assigned to receive tablets, but this effect was not found to be significant in the previously developed model. Despite the slightly higher dose, the box plots illustrate the considerable overlap in AUC₀₋₂₄ values between those predicted for children on once daily regimens and those observed in prior studies of adults and children; therefore, a once daily abacavir regimen is expected to have similar antiviral activity to the twice daily regimen in children. As shown in figures 2 and 3, abacavir simulated peak concentrations appear to be comparable to or higher than historical values, especially those of twice daily regimens. Given that once daily abacavir was approved for use in adults based on good safety and efficacy and the positive benefit-risk balance and acceptable safety profile of once daily abacavir was observed in small studies of children (19-21), the predicted increase in Cmax after once daily administration is unlikely to result in a higher risk of adverse events.

The higher plasma Cmax with the once daily regimen may raise questions about the potential for increased toxicity and adverse events in children. Abacavir has been studied at doses as high as 12mg/kg twice daily in children (31) and at doses up to 600mg three times daily in clinical trials in adults (32). Possible trends in adverse event incidence were observed for nausea, malaise, and gastrointestinal discomfort in these high dose studies. However, Cmax values from pediatric subjects on once daily dose regimens in PENTA 13, PENTA 15 or ARROW were similar to those in the high dose adult studies and no new safety concerns were raised in PENTA 13, PENTA 15 or ARROW suggesting that abacavir is relatively safe and well-tolerated at higher doses and Cmax values in children

Limitations

One of the main limitations of our investigation was the use of abacavir plasma pharmacokinetics, which can only be considered as a limited marker of drug exposure as it is the intracellular abacavir triphosphate metabolite that becomes pharmacologically active. However, given that adequate sampling for determination of intracellular concentrations of nucleoside triphosphate inhibitor triphosphates is logistically and technically difficult and the volume of blood needed to measure intracellular abacavir concentrations with current technology makes serial evaluations impractical for paediatric patients, abacavir plasma pharmacokinetics can be considered as an appropriate marker in children.

Conclusions

We have demonstrated that abacavir pharmacokinetics after once daily dosing is comparable to abacavir pharmacokinetics following twice a day administration. Abacavir AUC_{0-24} was simulated in a hypothetical paediatric population and appeared to be comparable to historical values of abacavir AUC_{0-24} from previous clinical studies in adults and children for every weight range. Therefore, comparable antiviral activity would be expected on a once daily abacavir regimen. Simulated C_{max} was found to be comparable or higher than historical values. The increase in C_{max} does not constitute a risk of toxicity given the safe and tolerable abacavir profile (30, 31).

This work is supportive of previous clinical studies which showed the feasibility of once daily dosing of abacavir in children. A model-based approach enabled the evaluation of an alternative dosing regimen in a large population of children. Based on these findings we can conclude that once daily dosing abacavir to HIV-infected children provides appropriate drug exposure and does not lead to risk of under dosing. Abacavir once daily regimen may therefore be used to improve adherence and provide optimal efficacy in paediatric antiretroviral combination therapy.

References

1. Simoni, J.M., Montgomery, A., Martin, E., New, M., Demas, P.A. & Rana, S. Adherence to antiretroviral therapy for pediatric HIV infection: a qualitative systematic review with recommendations for research and clinical management. *Pediatrics* **119**, e1371-83 (2007).
2. Hazra, R., Siberry, G.K. & Mofenson, L.M. Growing up with HIV: children, adolescents, and young adults with perinatally acquired HIV infection. *Annual review of medicine* **61**, 169-85 (2010).
3. Bangsberg, D.R., Moss, A.R. & Deeks, S.G. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. *The Journal of antimicrobial chemotherapy* **53**, 696-9 (2004).
4. Feingold, A.R., Rutstein, R.M., Meislich, D., Brown, T. & Rudy, B.J. Protease inhibitor therapy in HIV-infected children. *AIDS patient care and STDs* **14**, 589-93 (2000).
5. Flynn, P.M. *et al.* Virologic and immunologic outcomes after 24 weeks in HIV type 1-infected adolescents receiving highly active antiretroviral therapy. *The Journal of infectious diseases* **190**, 271-9 (2004).
6. Wiener, L., Riekert, K., Ryder, C. & Wood, L.V. Assessing medication adherence in adolescents with HIV when electronic monitoring is not feasible. *AIDS patient care and STDs* **18**, 527-38 (2004).
7. Anderson, P.L. Pharmacologic perspectives for once-daily antiretroviral therapy. *The Annals of pharmacotherapy* **38**, 1924-34 (2004).
8. Back, D. *et al.* Therapeutic drug monitoring in HIV infection: current status and future directions. *AIDS (London, England)* **16 Suppl 1**, S5-S37 (2002).
9. Somboonwit, C., Kurtyka, D. & Velez, A.P. Abacavir and lamivudine combination. *Expert opinion on drug metabolism & toxicology* **5**, 1599-606 (2009).
10. Rizzardini, G. & Zucchi, P. Abacavir and lamivudine for the treatment of human immunodeficiency virus. *Expert opinion on pharmacotherapy* **12**, 2129-38 (2011).
11. Dando, T.M. & Scott, L.J. Abacavir plus lamivudine: a review of their combined use in the management of HIV infection. *Drugs* **65**, 285-302 (2005).
12. Hervey, P.S. & Perry, C.M. Abacavir: a review of its clinical potential in patients with HIV infection. *Drugs* **60**, 447-79 (2000).

13. Achenbach, C.J., Scarsi, K.K. & Murphy, R.L. Abacavir/lamivudine fixed-dose combination antiretroviral therapy for the treatment of HIV. *Advances in therapy* **27**, 1-16 (2010).
14. Yuen, G.J., Weller, S. & Pakes, G.E. A review of the pharmacokinetics of abacavir. *Clinical pharmacokinetics* **47**, 351-71 (2008).
15. Faletto, M.B., Miller, W.H., Garvey, E.P., St Clair, M.H., Daluge, S.M. & Good, S.S. Unique intracellular activation of the potent anti-human immunodeficiency virus agent 1592U89. *Antimicrobial agents and chemotherapy* **41**, 1099-107 (1997).
16. Moyle, G. *et al.* Steady-state pharmacokinetics of abacavir in plasma and intracellular carbovir triphosphate following administration of abacavir at 600 milligrams once daily and 300 milligrams twice daily in human immunodeficiency virus-infected subjects. *Antimicrobial agents and chemotherapy* **53**, 1532-8 (2009).
17. Hawkins, T., Veikley, W., St Claire, R.L., Guyer, B., Clark, N. & Kearney, B.P. Intracellular pharmacokinetics of tenofovir diphosphate, carbovir triphosphate, and lamivudine triphosphate in patients receiving triple-nucleoside regimens. *Journal of acquired immune deficiency syndromes (1999)* **39**, 406-11 (2005).
18. Harris, M., Back, D., Kewn, S., Jutha, S., Marina, R. & Montaner, J.S.G. Intracellular carbovir triphosphate levels in patients taking abacavir once a day. *AIDS (London, England)* **16**, 1196-7 (2002).
19. Paediatric European Network for Treatment of AIDS (PENTA). Pharmacokinetic study of once-daily versus twice-daily abacavir and lamivudine in HIV type-1-infected children aged 3-<36 months. *Antiviral therapy* **15**, 297-305 (2010).
20. Bergshoeff, A. *et al.* Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
21. Musiime, V. *et al.* Pharmacokinetics and acceptability of once- versus twice-daily lamivudine and abacavir in HIV type-1-infected Ugandan children in the ARROW Trial. *Antiviral therapy* **15**, 1115-24 (2010).
22. Kline, M.W. *et al.* A phase I study of abacavir (1592U89) alone and in combination with other antiretroviral agents in infants and children with human immunodeficiency virus infection. AIDS Clinical Trials Group 330 Team. *Pediatrics* **103**, e47 (1999).
23. Hughes, W. *et al.* Safety and single-dose pharmacokinetics of abacavir (1592U89) in human immunodeficiency virus type 1-infected children. *Antimicrobial agents and chemotherapy* **43**, 609-15 (1999).
24. GlaxoSmithKline Document Number GM1997/00142/00. Study ID CNAA2001. Multiple-dose pharmacokinetics and pharmacodynamics of abacavir alone and in combination with zidovudine in human immunodeficiency virus-infected adults.
25. GlaxoSmithKline Study ID CAL10001. An evaluation of the bioequivalence of a combined formulated tablet (600mg/300mg abacavir/lamivudine) compared to ZIAGEN† (abacavir) 2 X 300mg tablets and EPIVIR† (lamivudine) 2 X 150mg tablets administered concurrently and the effect of food on absorption of the combined formulation in healthy adult subjects.
26. GlaxoSmithKline Study ID CAL102120. An open-label, two-period, crossover, pharmacokinetic study of abacavir and its intracellular anabolite carbovir triphosphate following once-daily and twice-daily administration of abacavir in HIV-infected subjects.
27. Zhao, W., Piana, C., Danhof, M., Burger, D., Pasqua, O.D. & Jacqz-Aigrain, E. Population pharmacokinetics of abacavir in infants, toddlers and children. *British journal of clinical pharmacology* **75**(6), 1525-35 (2013)
28. The WHO Child Growth Standards. Available at: <http://www.who.int/childgrowth/en/> (last accessed 3 June 2013)
29. Summary of Product Characteristics of abacavir. Available at: <http://apps.who.int/prequal/whopar/whoparproducts/HA371part4v1.pdf> (last accessed 3 June 2013)
30. Bellanti, F. & Della Pasqua, O. Modelling and simulation as research tools in paediatric drug development. *European journal of clinical pharmacology* **67** Suppl 1, 75-86 (2011).
31. Saavedra-Lozano, J. *et al.* Salvage therapy with abacavir and other reverse transcriptase inhibitors for human immunodeficiency-associated encephalopathy. *The Pediatric infectious disease journal* **25**, 1142-52 (2006).
32. McDowell, J.A., Lou, Y., Symonds, W.S. & Stein, D.S. Multiple-dose pharmacokinetics and pharmacodynamics of abacavir alone and in combination with zidovudine in human immunodeficiency virus-infected adults. *Antimicrobial agents and chemotherapy* **44**, 2061-7 (2000).

SECTION IV

Forgiveness to poor adherence

FORGIVENESS OF NON-ADHERENCE FOR DIFFERENT CLASSES OF ANTIRETROVIRAL DRUGS IN CHILDREN

Chiara Piana, Sean Oosterholt, Meindert Danhof, Oscar Della Pasqua

Submitted for publication

SUMMARY

The aim of this investigation was to evaluate the forgiveness of antiretroviral therapy to variable adherence, taking into account the differences in pharmacokinetic and pharmacodynamic properties of the drugs currently administered to HIV-infected children as first-line therapy.

Simulation scenarios were evaluated using a hypothetical population of HIV-infected children ($n=100$) between 3 and 12 years. Three drugs, belonging to the three antiretroviral classes approved as first-line therapy in children, were selected: efavirenz (NNRTI), lamivudine (NRTI) and lopinavir/ritonavir (boosted PI). Published pharmacokinetic and pharmacodynamic models were integrated with an established model for viral replication to predict treatment outcome based on different degrees of adherence to therapy for each drug. Simulations were performed in NONMEM7 and R 2.13.0 was used for data manipulation, statistical and graphical summaries.

Despite its long half-life, efavirenz may be susceptible to viral failure for treatment interruptions longer than one week. Due to its short half-life, lamivudine appears to be forgiving only to very short periods and few missed doses. Similarly, forgiveness of non-adherence to treatment with lopinavir/ritonavir is limited to short treatment interruptions and few missing doses. Based on the current dosing regimens, no relevant clinical effect is observed for delays in drug intake of up to six hours for the three drugs.

Our results show that simulations can be applied as a tool to explore non-adherence to treatment. The use of a model-based approach provides a framework for the optimisation of the dosing regimens for antiretroviral drugs, unravelling the set of pharmacokinetic and pharmacodynamic properties that determine forgiveness.

8.1. INTRODUCTION

The goal of antiretroviral therapy is to delay disease progression by minimizing viral replication and preserving immune function, with minimal drug-toxicity effects. Several antiretroviral drugs are currently approved in children. They are classified by their target in the HIV life-cycle as nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) (1). Yet, children with HIV represent a highly complex population. In fact, despite the available drugs, viral failure remains a serious issue in paediatric HIV. A previous study has shown that 24% of children followed up over 4 years were triple-class exposed, and 10% had triple-class viral failure (2).

These failure rates may be explained by the chronic nature of the disease, which requires the use of drug combinations throughout adolescence and adulthood. During growth, time varying developmental factors that affect the pharmacokinetics of antiretroviral drugs are often ignored, which leads to inappropriate exposure levels. Secondly, young children must rely on a caregiver to receive their medications. Given the social and economic constraints, caregivers may be busy during the day, causing dosing frequency to be variable and making adherence to treatment rather challenging. Although simplified treatments are highly advantageous, currently only few antiretroviral medications are available as a once daily dosing regimen in children. Indeed, fewer than 50% of the children/caregivers report an adherence of 100% to their treatment (3).

Reasons of non-adherence include: forgetting doses, changes in routine, being too busy, and child refusal. In some cases, however, the cause of poor adherence is unrelated to the patient; it is due to the environment: patients in limited resource countries do not always have access to antiviral drugs as drug supplies may be limited or they cannot afford the cost of treatment. Therefore, even when a child receives the optimal dose and has access to simplified dosing regimens, the impact of poor adherence to treatment still needs to be considered carefully: compliance to the prescribed regimen is very difficult to achieve in practice. It has been demonstrated that the risk of viral failure increases as the amount of missed doses increases (4–6). Imperfect adherence can lead to sub-therapeutic drug levels, which may boost the development of drug resistance to one or several drugs in the treatment. In fact, it is estimated that a degree of adherence equal or higher than 95% is required for a successful viral treatment (95 rule) (7). More recent studies with new classes of antiretroviral drugs show that a degree of adherence lower than 95% may be sufficient to maintain viral suppression, but these findings result from empirical experimental protocols and as such may not be generalisable (8).

Given that HIV therapy is usually a lifelong treatment it is critical to understand whether a drug allows for such patterns of poor adherence. The objective of our analysis is therefore to investigate the forgiveness of non-adherence of each class of antiretroviral drugs currently used as first-line therapy in children based on their pharmacokinetic and pharmacodynamic

properties such as half-life and potency. For the sake of clarity, the forgiveness of a drug is defined as the ability to achieve and maintain viral suppression despite sub-optimal adherence to the prescribed dosing regimen. This may depend on many factors, such as drug, viral and host properties. Forgiveness of non-adherence may be a powerful factor in the selection of the most suitable treatment in resource limited countries.

In contrast to empirical protocols, here we propose a model-based approach to evaluate forgiveness of drug to treatment interruptions and deviations from the prescribed regimen, which cannot be assessed in a real-life randomised controlled trial due to obvious ethical and clinical reasons. We assess adherence as a covariate effect on drug exposure using a range of scenarios. Similar approaches dealing with adherence have been proposed before (9). However, an integrated analysis of the impact of adherence on pharmacodynamics has not been performed so far. Instead, Markov models have been developed to estimate the effect of patient adherence on the rate at which patient progress through the HIV infection (10–12). More recently, Genberg *et al* (13) have shown the implication of treatment interruptions on the outcome of antiretroviral therapy, but their work has not explored how pharmacokinetic-pharmacodynamic properties contribute to or prevent treatment failure.

8.2. METHODS

Hypothetical population and paradigm drugs

A hypothetical population consisting of 100 HIV-infected between 3 and 12 years old was selected with individuals evenly spread across two groups based on body weight (15–40kg and >40kg). Three paradigm drugs were evaluated as monotherapy: efavirenz (NNRTI), lamivudine (NRTI) and boosted lopinavir/ritonavir (boosted PI). The drugs were selected to represent the antiretroviral classes presently approved as first-line therapy in children (6). Each drug was administered according to the currently recommended dose (table 1), as indicated in the latest Summary of Product Characteristics. The duration of the treatment in this hypothetical trial was 90 days. Viral load was considered the measure of interest in the study. Further details of the proposed experimental protocol are summarised in table 2.

Models for drug pharmacokinetics, drug resistance and drug efficacy

In order to explore the effect of a range of sub-optimal adherence scenarios on treatment outcome, including that of drug holidays, three mathematical models were used for simulation purposes during the analysis. First a pharmacokinetic model was used to predict the time course of plasma concentrations for each drug. Subsequently a pharmacodynamic model was applied to predict the time varying inhibitory effect of the drugs and ultimately a disease model for viral replication and infection was employed to predict the time varying clinical endpoints (viral load).

Table 1 Doses of antiretroviral drugs administered to the hypothetical population of HIV-infected children based on current guidelines

Antiretroviral drug	Dosing regimen
Efavirenz	250 mg once daily (14-20 kg) 300 mg once daily (20-25 kg) 350 mg once daily (25-32 kg) 400 mg once daily (32-39 kg) 600 mg once daily (>39 kg)
Lamivudine	150 mg twice daily (14-21 kg) 225 mg twice daily (21-30 kg) 300 mg twice daily (>30 kg)
Boosted lopinavir/ritonavir	15/40 mg/Kg twice daily (15-40 kg) 400/100 mg twice daily (>40 kg)

Table 2 Details of the hypothetical clinical protocol

	EFV	3TC	LPV/r
Number of virtual patients	100	100	100
Treatment duration (days)	90	90	90
Dosing frequency	Once daily	Twice daily	Twice daily
Sampling frequency	Daily	Daily	Daily
Assumed failure time (days)	40	40	40

The pharmacokinetic profiles over time for each drug were simulated based on validated models available in literature (14, 15).

The median inhibitory concentration (IC_{50}) was used to quantify agent-specific drug susceptibility. To model within-host changes over time in IC_{50} attributable to the emergence of new-drug resistant mutations the function depicted in equation (1) was used (16).

$$IC_{50}(t) = \begin{cases} I_0 + \frac{I_r - I_0}{t_r} t & \text{for } 0 < t < t_r \\ I_r & \text{for } t \geq t_r \end{cases} \quad (1)$$

where I_0 and I_r are respective values of $IC_{50}(t)$ at baseline and at time point t_r at which the resistant mutations dominate. If $I_r = I_0$, no new drug resistant mutation is developed during treatment. More complicated models for IC_{50} are available in literature (17, 18); however in clinical practice it is common to collect IC_{50} values only at baseline and failure time (19).

In our investigation the failure time was fixed at 40 days after the start of the treatment given that when antiretroviral drugs are administered in monotherapy resistance usually develops within few weeks after the start of treatment. The values of IC_{50} for the drugs explored in this analysis were obtained from literature (20–22), such as the values of I_r based on phenotypic resistance tests for each drug.

Efficacy was considered to be constant over time in several viral dynamic studies (23–25). However, treatment response may vary because of differences in drug concentration and other factors or conditions (i.e. development of resistance), which may change during treatment. A pharmacodynamic additive sigmoid I_{max} model was used to describe the concentration-effect relationship:

$$I = \frac{I_{max}C}{IC_{50} + C} \quad (2)$$

where I_{max} is the maximal inhibitory effect that can be achieved and equals to 1, C is the drug concentration, and IC_{50} is the drug concentration that induces an effect equivalent to 50% of the maximal inhibitory effect.

Given that during our analysis the three drugs were administered as monotherapy, the time-varying efficacy or response to treatment equals the effect of the drug at each time point, as calculated in equation 2. Time-varying efficacy $\gamma(t)$ ranges from 0 to 1 and is referred to as the drug efficacy index (the inhibition rate of viral replication) in the viral dynamic model (Equation 3-5). If $\gamma(t) = 1$, the drug is 100% effective, whereas if $\gamma(t) = 0$, the drug has no effect.

Mathematical models for HIV dynamics

A widely used model for viral infection and replication was used to simulate the time course of the clinical endpoints (viral load and CD4 count) (19). As depicted in figure 1, the model consists of 3 compartments: uninfected target cells (T), free virions (V) and infected target cells (T^*). Although more complex models for viral replication were available in literature, this simple model showed characteristics of stability and robustness deemed suitable for the purpose of our analysis.

The model for viral replication is defined by three differential equations (equation 3-5). Several parameters are required to describe the viral and immunological processes: λ ($\text{day}^{-1} \text{mm}^{-3}$) is the birth rate of new T cells appearing in the body, d_T (day^{-1}) is the death rate of the T cells, N is the amount of virions produced by each of the infected target cells, c (day^{-1}) is the clearance of the free virions, k ($\text{day}^{-1} \text{mm}^3$) is the infection rate, δ (day^{-1}) is the death rate of infected target cells; $\gamma(t)$ is the time varying efficacy of the drug, predicted by the pharmacodynamic model depicted in equation (2).

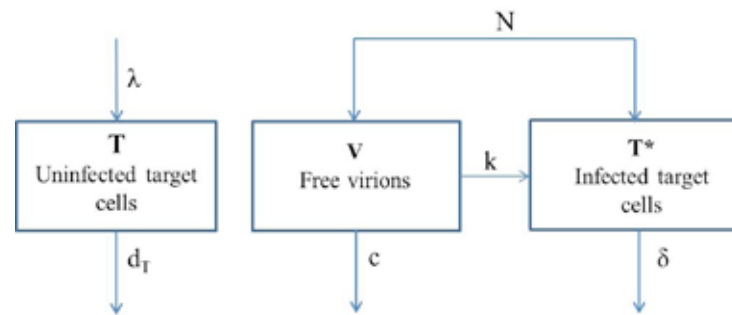


Figure 1 Three-compartment model for viral replication and infection used to simulate time-varying viral load and CD4 count.

$$dT / dt = \lambda - d_T - [1 - \gamma(t)] kTV \quad (3)$$

$$dT^* / dt = [1 - \gamma(t)] kTV - \delta T^* \quad (4)$$

$$dV / dt = N\delta T^* - cV \quad (5)$$

Simulation scenarios of variable adherence to treatment

The diagram in figure 2 depicts all the scenarios which were simulated for different patterns of non-adherence to treatment (treatment interruption, delay in drug intake or single doses randomly missed throughout the clinical trial).

Based on average adherence in clinical trials derived from objective measures (4, 26, 27), a pre-defined fraction of patients (40%) was randomly selected to be non-adherent to the prescribed dosing regimen. For each scenario the period of non-adherence was randomly assigned to each subject across the duration of the trial as shown in figure 3. 100 replicates were simulated for each scenario. Simulations were performed with NONMEM v7 and R (v 2.13.0) was used to produce the datasets for each simulation scenario and to numerically and graphically summarise the results. A comparison between treatment outcome for the full population in case of perfect and

imperfect adherence was made to evaluate the forgiveness of non-adherence for each drug and explore which drug-specific properties have to be considered to achieve viral success.

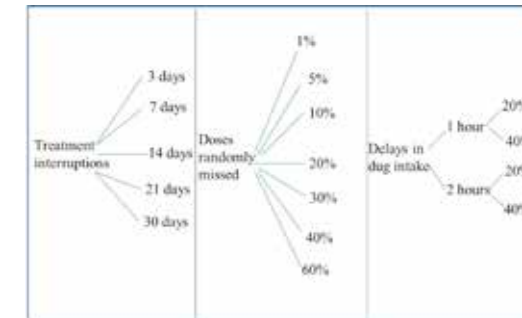


Figure 2 Diagram depicting the simulation scenarios of non-adherence to treatment investigated during the analysis.

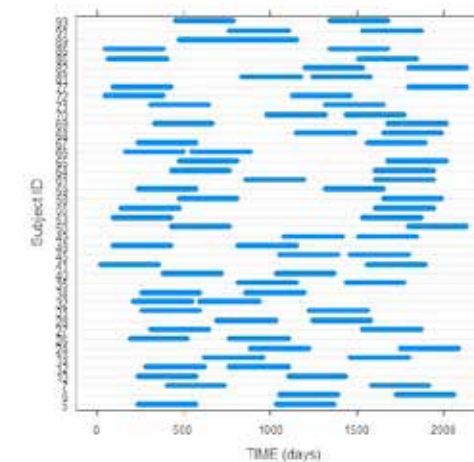


Figure 3 Example of randomly assigned periods of non-adherence for one subject (treatment interruption of 14 days (7x2)).

Pharmacodynamic vs. clinical forgiveness

Given the lack of quantitative measures to characterise forgiveness of non-adherence, two thresholds were defined in our study. The first threshold corresponds to an increase of 20% in the median value of viral load compared to perfect adherence. Such a threshold, which indicates a variation in drug response, was selected to account for the inter-individual variability defined in the models. If the viral load is below this threshold, we consider that the drug allows for pharmacodynamic forgiveness.

The second threshold corresponds to an increase in median viral load equal to 0.5 log10 copies/mL. Such an increase, which was selected to account for assay variability (28), can be considered clinically relevant and indicate viral failure. If the viral load is below this threshold we consider that the drug allows for clinical forgiveness.

Two main assumptions were required to implement the simulations presented here. First of all, we have assumed the same failure time for the three drugs (day 40). This was done to avoid additional variability in the study and evaluate adherence as the only variable in our analysis. Secondly, the selection of discrete scenarios of non-adherence was deemed necessary to evaluate the forgiveness of each drug. Nevertheless, these scenarios reflect the most common patterns of non-adherence and were defined according to a more detailed taxonomy for compliance, based upon which treatment interruptions, random missingness of doses or delays in drug intake can be characterised.

8.3. RESULTS

Viral load and CD4 counts were derived by simulations for all three drugs. Given that both end-points appeared to be equally affected by varying patterns of compliance, only viral load data will be presented and summarised in the next paragraphs.

The boxplots in figure 4-6 show the median viral load for the children non-adherent to therapy (n=40) for each simulated scenario of non-adherence at the last time point before resistance dominates. After this time point the treatment is no longer effective due to resistance. As indicated previously, the evaluation of the effects of poor adherence according to a model-based approach requires a more detailed taxonomy. Therefore, our results will be presented in terms of treatment interruptions, random missingness and delays in drug intake.

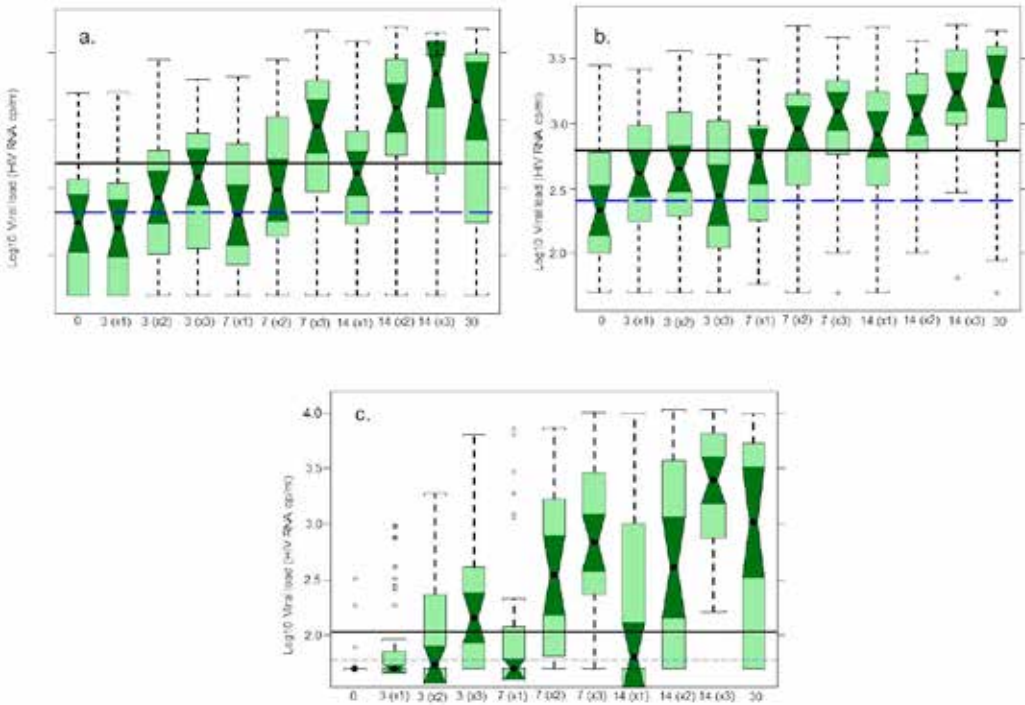


Figure 4 Boxplots show the implications of poor adherence on viral load prior to drug failure (i.e. resistance) for variable persistence of compliance. On the x-axis the duration of the treatment interruption is displayed. In parenthesis the number of interruptions is illustrated. The lines represent two thresholds for variation in response assuming perfect adherence to treatment: the dashed line indicates the threshold for pharmacodynamic forgiveness; the solid line indicates the threshold for clinical forgiveness.
a. efavirenz, b. lamivudine, c. lopinavir/ritonavir

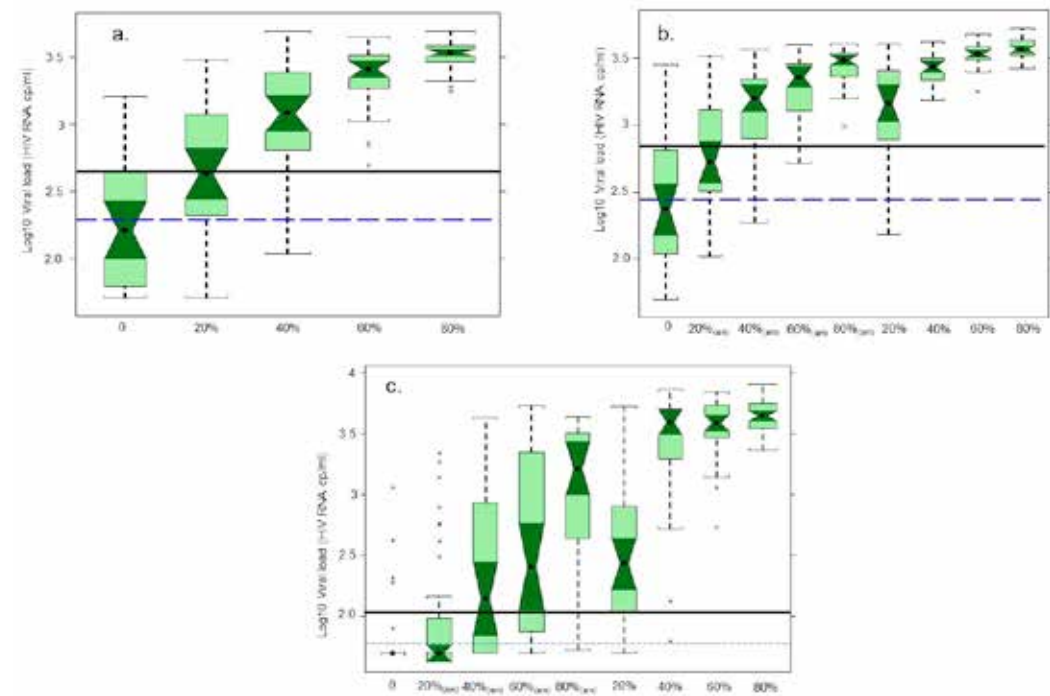


Figure 5 Boxplots show the implications of poor adherence on viral load prior to drug failure (i.e. resistance) for randomly missed doses. On the x-axis the percentage of doses missed is displayed. For lamivudine and lopinavir/ritonavir, administered twice daily, the scenarios in which only the morning dose is missed are illustrated as well. The lines represent two thresholds for variation in response assuming perfect adherence to treatment: the dashed line indicates the threshold for pharmacodynamic forgiveness; the solid line indicates the threshold for clinical forgiveness.

a. efavirenz, b. lamivudine, c. lopinavir/ritonavir

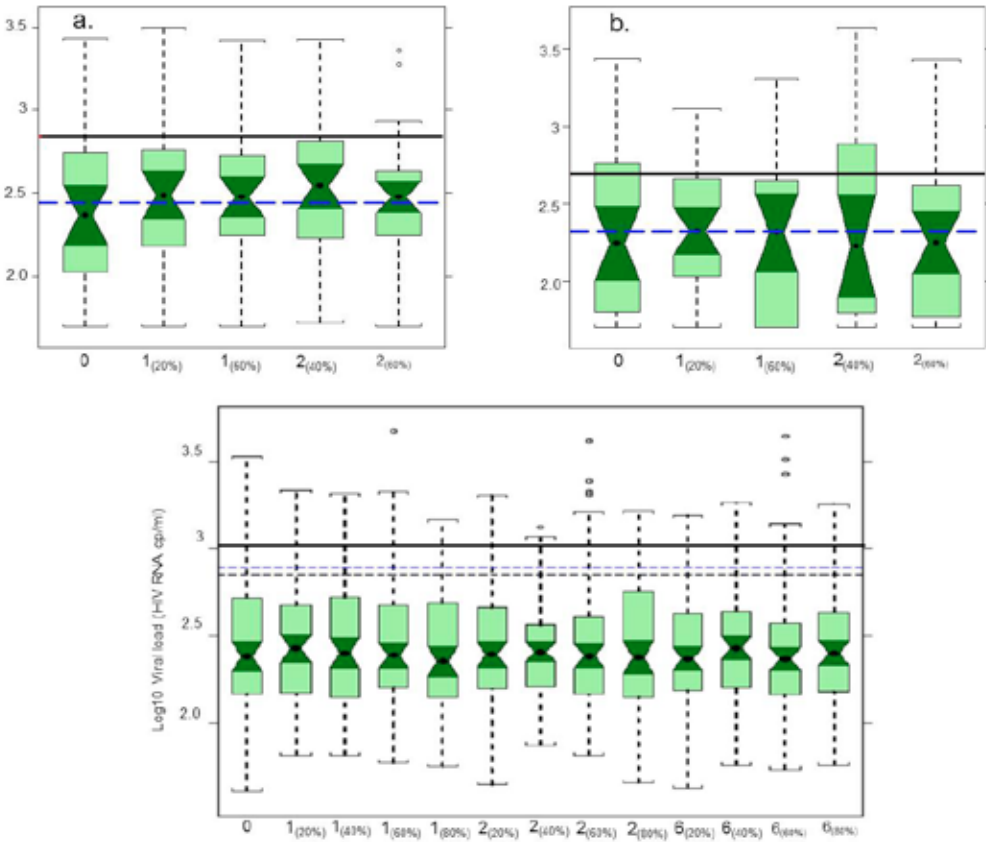


Figure 6 Boxplots show the implications of poor adherence on viral load prior to drug failure (i.e. resistance) for variable quality of compliance. On the x-axis the number of hours of delay in drug intake is depicted. In parenthesis the percentage of days of treatment in which the delay happens is displayed. The lines represent two thresholds for variation in response assuming perfect adherence to treatment: the dashed line indicates the threshold for pharmacodynamic forgiveness; the solid line indicates the threshold for clinical forgiveness. The lower dashed line in panel c represents the quantification limit for viral load.

a. efavirenz, b. lamivudine, c. lopinavir/ritonavir

Treatment interruptions

Median viral load values are presented separately for each of the three drugs. In figure 4 an increase in median viral load is clearly visible between scenarios with variable treatment interruptions. However, significant differences in the effect on poor adherence can be observed for the different drugs. Median viral load values above the threshold of clinical forgiveness are reached for efavirenz after repeated treatment interruptions of one week (7x3) or two weeks (14x2). On the other hand less frequent treatment interruptions (7x2) for lamivudine and boosted lopinavir/ritonavir are sufficient to raise median values of viral load. Moreover, our analysis shows that repeated treatment interruptions of three days do not seem to alter treatment response for efavirenz, while similar interruptions may lead to treatment failure for lamivudine and lopinavir/ritonavir.

Doses randomly missed

Different patterns of response were observed for each of the drugs when doses are missed randomly, as shown in figure 5. Missing a dose twice a day for 20% of the days of treatment and missing one dose a day for 40% of the days of treatment (the amount of total doses missed is the same) leads to different median viral load values but overlapping confidence interval. In the case of efavirenz, viral failure may be caused by 40% of the doses randomly missed during the trial, whilst for lamivudine and boosted lopinavir/ritonavir 20% of the daily dose or 40% of half-daily dose missed may cause viral failure. In addition, we have found that the median viral load increases as the amount of missed doses increases. The difference between missing a dose once or twice a day appears to be important. For lopinavir/ritonavir when a dose is missed once a day for 20% of the days of treatment the median viral load does not reach the threshold of pharmacodynamic forgiveness while missing a dose twice a day for 20% of the days causes an increase in median viral load above the threshold for clinical forgiveness.

Delays in drug intake

Based on the box plots in figure 6, no variations were observed in median viral load for the three drugs for delays in drug intake up to six hours. Although the 10th and 90th percentiles show small changes, the median values of viral load for efavirenz, lamivudine and boosted lopinavir/ritonavir remain very similar to the value observed in the case of perfect adherence for each scenario of delay in drug intake. Given that median viral load values for lopinavir/ritonavir stay below the limit of quantification for all the scenarios, in figure 6 c the values of viral load below the quantification limit are also shown. Such values can be predicted by the model but are not quantifiable in clinical practice.

8.4. DISCUSSIONS AND CONCLUSIONS

Using a model-based approach, we have shown that integration of pharmacokinetic and pharmacodynamic models to a disease model for viral replication and infection can provide the basis for a framework for the evaluation of patient behaviour during a clinical trial. Our analysis clearly shows a relationship between the duration of treatment interruption and increase in viral load for the three classes of antiretroviral drugs evaluated. For NNRTIs the risk for viral failure is statistically significant starting at interruptions between 7 and 14 days, whilst for PI-based regimens, the risk of viral failure is statistically significant at 2–7 days. This findings are in agreement with a previous investigation which shows the impact of treatment interruptions on treatment outcome (13).

Despite its long half-life (40–55 hours), efavirenz seems to be completely forgiving only to very short interruptions. Longer or repeated interruptions may lead either to change in treatment outcome or to viral failure, depending on the duration and frequency of such interruptions. These findings are in agreement with previous publications which show that, despite its pharmacokinetic and pharmacodynamic properties, high levels of adherence to NNRTIs are needed to control viral replication (5, 29).

Published data also confirm our findings with boosted PIs. According to these studies, an adherence rate of at least 80% is required to achieve viral suppression (30–34). In addition, longer half-life and increased PI concentrations produced by the boosting effect of ritonavir can increase the forgiveness of PIs. However, higher levels of adherence are needed to the boosted PIs compared with the NNRTIs in order to obtain the same virologic suppression.

Very little information was available in literature about the impact of non-adherence to NRTIs. Based on our findings, lamivudine seems to be less forgiving than efavirenz due to its shorter half-life. Yet, despite its pharmacokinetic profiles, delays in drug intake do not seem to affect treatment outcome (as depicted in figure 6 b).

Clinical relevance

From a clinical perspective, knowledge of the level of adherence necessary to achieve virologic suppression can be extremely valuable as it may help to determine when adherence counselling is most necessary as well as whether alternative antiretroviral drugs need to be administered given a patient's adherence pattern (30). Furthermore, quantitative measures of forgiveness of non-adherence and class-specific thresholds may represent a powerful tool for patients and clinicians. In fact, we envisage that the availability of a methodology that allows correlation of the forgiveness to the pharmacokinetic and pharmacodynamic properties of a drug may be used during the development of novel antiretroviral drugs and in the evaluation of the robustness of existing drugs not yet approved in children.

Furthermore, it may be used to explore the behaviour of new antiretroviral compounds in children for which little empiric data exists.

We reiterate that the importance of our analysis relies mainly on the fact that the use of a model-based approach allows one to evaluate critical scenarios which cannot be investigated in real-life or controlled in a randomised protocol. In fact, it appears to be the only way to generate drug-specific thresholds of forgiveness of non-adherence. In conjunction with simulation techniques, this approach can provide the basis for improved dose rationale and better dosing regimens in children.

From a drug development perspective, the integration of pharmacokinetic-pharmacodynamic models with a viral dynamics model also offers an opportunity to predict the efficacy of antiretroviral combinations. Estimates of pharmacokinetic-pharmacodynamic relationships in vivo are often challenging because of the use of combination therapy, which prevents detailed evaluation of the contribution of each drug to the overall inhibitory effect on viral load. Yet, scenarios can be considered in which drug effects are explored under the assumption of additivity and taking into account differences in pharmacokinetics (35).

Limitations in our approach

We are aware that the clinical setting depicted in our simulation exercise does not fully reflect clinical practice, in that each drug is not administered as monotherapy. Antiretroviral drugs have been always administered in combination. In fact, monotherapy has been avoided mainly because of the rapid development of drug resistance. However this setting was required to investigate which properties determine the forgiveness of non-adherence to an antiretroviral drug. On the other hand, we have considered the implications of drug resistance, which has been observed for monotherapy with antiretroviral drugs. However, we have not been able to identify a clear relationship between poor adherence and increased probability to develop drug resistance, particularly for NNRTIs. In fact, it has been shown that despite their long half-life, such drugs may remain for long time at sub-therapeutic levels, which may lead to a higher risk of resistance.

Given that our primary aim was to explore which pharmacological properties determine forgiveness, we do not anticipate any bias due to this limitation. We acknowledge however that the inclusion of a correlation between non-adherence and development of resistance may improve the assessment of the time to viral failure and consequently the impact of poor adherence.

Conclusions

In summary, we have evaluated the impact of non-adherence for three drugs used in first line therapy in children. Undoubtedly, our methodology offers the opportunity to explore the liability of novel compounds to variable patterns of drug intake, which are often unfeasible in experimental protocols. Although generalisation of the findings cannot be warranted without further investigations, we envisage that other mechanisms and drug combinations can be evaluated in a similar manner. In fact, the use of a model-based approach may provide a framework for the optimisation of the dosing regimens in paediatric HIV.

References

1. Antiretroviral therapy for HIV infection in infants and children. Available at: <http://www.who.int/hiv/pub/paediatric/paed-prelim-summary.pdf> (last accessed 12 June 2013)
2. Castro, H. *et al.* Risk of triple-class virological failure in children with HIV: a retrospective cohort study. *Lancet* **377**, 1580-7 (2011).
3. Van Dyke, R.B., Lee S., Johnson G.M. *et al.* Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. *Pediatrics* **109**(4), e61 (2002).
4. Arnsten, J.H. *et al.* Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **33**, 1417-23 (2001).
5. Nachega, J.B., Hislop, M., Dowdy, D.W., Chaisson, R.E., Regensberg, L. & Maartens, G. Adherence to nonnucleoside reverse transcriptase inhibitor-based HIV therapy and virologic outcomes. *Annals of internal medicine* **146**, 564-73 (2007).
6. WHO Pediatric guidelines Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents* **5**, 79-104 (2012).
7. Bangsberg, D.R. *et al.* Non-adherence to highly active antiretroviral therapy predicts progression to AIDS. *AIDS (London, England)* **15**, 1181-3 (2001).
8. Bangsberg, D.R. Less than 95% adherence to nonnucleoside reverse-transcriptase inhibitor therapy can lead to viral suppression. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **43**, 939-41 (2006).
9. Pfister, M. *et al.* Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group Study 398. *Antimicrobial agents and chemotherapy* **47**, 130-7 (2003).
10. Vrijens, B., Goetghebeur, E., de Klerk, E., Rode, R., Mayer, S. & Urquhart, J. Modelling the association between adherence and viral load in HIV-infected patients. *Statistics in medicine* **24**, 2719-31 (2005).

11. Labbé, L. & Verotta, D. A non-linear mixed effect dynamic model incorporating prior exposure and adherence to treatment to describe long-term therapy outcome in HIV-patients. *Journal of pharmacokinetics and pharmacodynamics* **33**, 519-42 (2006).
12. Girard, P., Blaschke, T.F., Kastrissios, H. & Sheiner, L.B. A Markov mixed effect regression model for drug compliance. *Statistics in medicine* **17**, 2313-33 (1998).
13. Genberg, B.L. et al. Patterns of antiretroviral therapy adherence and impact on HIV RNA among patients in North America. *AIDS (London, England)* **26**, 1415-23 (2012).
14. Dickinson, L. et al. Sequential population pharmacokinetic modeling of lopinavir and ritonavir in healthy volunteers and assessment of different dosing strategies. *Antimicrobial agents and chemotherapy* **55**, 2775-82 (2011).
15. Kappelhoff, B.S. et al. Population pharmacokinetics of efavirenz in an unselected cohort of HIV-1-infected individuals. *Clinical pharmacokinetics* **44**, 849-61 (2005).
16. Huang, Y., Rosenkranz, S.L. & Wu, H. Modeling HIV dynamics and antiviral response with consideration of time-varying drug exposures, adherence and phenotypic sensitivity. *Mathematical biosciences* **184**, 165-86 (2003).
17. Bonhoeffer, S., Lipsitch, M. & Levin, B.R. Evaluating treatment protocols to prevent antibiotic resistance. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12106-11 (1997).
18. Wainberg, M.A., Hsu, M., Gu, Z., Borkow, G. & Parniak, M.A. Effectiveness of 3TC in HIV clinical trials may be due in part to the M184V substitution in 3TC-resistant HIV-1 reverse transcriptase. *AIDS (London, England)* **10 Suppl 5**, S3-10 (1996).
19. Wu, H. et al. Modeling long-term HIV dynamics and antiretroviral response: effects of drug potency, pharmacokinetics, adherence, and drug resistance. *Journal of acquired immune deficiency syndromes (1999)* **39**, 272-83 (2005).
20. Best, B.M. et al. Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. *The Journal of antimicrobial chemotherapy* **66**, 354-7 (2011).
21. Fletcher, C.V. et al. Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. *AIDS (London, England)* **14**, 2137-44 (2000).
22. Melroy, J. & Nair, V. The antiviral activity, mechanism of action, clinical significance and resistance of abacavir in the treatment of pediatric AIDS. *Current pharmaceutical design* **11**, 3847-52 (2005).
23. Wein, L.M., D'Amato, R.M. & Perelson, A.S. Mathematical analysis of antiretroviral therapy aimed at HIV-1 eradication or maintenance of low viral loads. *Journal of theoretical biology* **192**, 81-98 (1998).
24. Wu, H. & Ding, A.A. Population HIV-1 dynamics in vivo: applicable models and inferential tools for virological data from AIDS clinical trials. *Biometrics* **55**, 410-8 (1999).
25. Ding, A.A. & Wu, H. Relationships between antiviral treatment effects and biphasic viral decay rates in modeling HIV dynamics. *Mathematical biosciences* **160**, 63-82 (1999).
26. Gross, R., Bilker, W.B., Friedman, H.M. & Strom, B.L. Effect of adherence to newly initiated antiretroviral therapy on plasma viral load. *AIDS (London, England)* **15**, 2109-17 (2001).
27. Liu, H. et al. A comparison study of multiple measures of adherence to HIV protease inhibitors. *Annals of internal medicine* **134**, 968-77 (2001).
28. Brambilla, D. et al. The contribution of assay variation and biological variation to the total variability of plasma HIV-1 RNA measurements. The Women Infant Transmission Study Clinics. Virology Quality Assurance Program. *AIDS (London, England)* **13**, 2269-79 (1999).
31. Saberi, P., Caswell, N., Amodio-Groton, M. & Alpert, P. Pharmacy-refill measure of adherence to efavirenz can predict maintenance of HIV viral suppression. *AIDS care* **20**, 741-5 (2008).
32. Kobin, A.B. & Sheth, N.U. Levels of Adherence Required for Virologic Suppression Among Newer Antiretroviral Medications. *Annals of Pharmacotherapy* **45**, 372-379 (2011).
33. Parienti, J.-J. et al. Average adherence to boosted protease inhibitor therapy, rather than the pattern of missed doses, as a predictor of HIV RNA replication. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **50**, 1192-7 (2010).
34. Shuter, J., Sarlo, J.A., Kanmaz, T.J., Rode, R.A. & Zingman, B.S. HIV-infected patients receiving lopinavir/ritonavir-based antiretroviral therapy achieve high rates of virologic suppression despite adherence rates less than 95%. *Journal of acquired immune deficiency syndromes (1999)* **45**, 4-8 (2007).
35. Martin, M. et al. Relationship between adherence level, type of the antiretroviral regimen, and plasma HIV type 1 RNA viral load: a prospective cohort study. *AIDS research and human retroviruses* **24**, 1263-8 (2008).

36. Nelson, M. *et al.* Suboptimal adherence to darunavir/ritonavir has minimal effect on efficacy compared with lopinavir/ritonavir in treatment-naïve, HIV-infected patients: 96 week ARTEMIS data. *The Journal of antimicrobial chemotherapy* **65**, 1505-9 (2010).
37. De Cock, R.F.W., Piana, C., Krekels, E.H.J., Danhof, M., Allegaert, K. & Knibbe, C.A.J. The role of population PK-PD modelling in paediatric clinical research. *European journal of clinical pharmacology* **67 Suppl 1**, 5-16 (2011).

IMPACT OF NON-ADHERENCE TO ANTIRETROVIRAL COMBINATION THERAPY IN HIV-INFECTED CHILDREN

Chiara Piana, Donato Teutonico, Meindert Danhof, Oscar Della Pasqua

SUMMARY

Sub-optimal adherence to therapy is among the main causes of failure in the treatment of HIV. In children such issue is particularly serious given that they rely on caregivers to receive their medications. The aim of this study is to evaluate the impact of different patterns of non-adherence for a widely used NNRTI-based regimen in children.

Clinical trial simulations (CTS) were performed in R 2.14. A hypothetical population of HIV-infected children between 3 and 12 years old was simulated ($n=100$). Published pharmacokinetic and pharmacodynamic models were integrated with an established model for viral replication to predict treatment outcome based on various degrees and different patterns of non-adherence to therapy. A logistic regression was used to incorporate the relation between sub-therapeutic drug levels and the probability of developing resistance. The duration of the hypothetical trial was 48 weeks and the primary efficacy endpoint was the proportion of patients with HIV-1 RNA <50 copies/mL at week 48.

Treatment interruptions of two weeks to NNRTI-based regimens may extensively increase the proportion of children experiencing viral failure compared to perfect adherence (76% vs. 90% of the children achieving a viral load <50 copies/mL at week 48), as well as 10% of doses randomly missed during the 48-weeks trial (78% vs. 90% of the children achieving viral load <50 copies/mL at week 48). Delays in drug intake up to 4 hours do not impact the outcome of the treatment.

Based on our findings, treatment interruptions to a NNRTI-based regimen may pose more risk for virologic rebound than the same number of randomly missed doses. Clinical trial simulations can be applied as a tool to explore the impact of different patterns of non-adherence to combination treatment in children, which could not be evaluated in clinical practice due to obvious ethical reasons.

9.1. INTRODUCTION

In the last decades considerable progress has been made in the delivery of antiretroviral therapy. Among other things, the use of fixed-dose combinations has been proven to be a powerful approach to improve patient adherence. However, these improvements are not always equally applicable to children, who will be subjected to lifelong treatment and who most likely will face the burden of disease in resource limited areas, where the access to the medication may be inadequate. Such challenges are further compounded by the fact that paediatric patients also have to rely on the availability of caregiver for the administration of the drugs.

As shown in previous studies, adherence is the strongest predictor of HIV-RNA suppression among individuals infected with HIV (1). In fact, many studies have been performed to investigate the role of adherence in HIV therapy, however in most cases adherence has been measured as the percentage of prescribed doses taken. Such a definition of adherence is outdated and has been shown to have important limitations (2). Today, it is acknowledged that differences in individual patterns of non-adherence can have different implications for the treatment outcome.

Thus far no quantitative assessment has been made of the patterns of non-adherence in children, in which the role of extrinsic factors and intrinsic properties of the drugs used in combination therapy are distinguished. Such data may allow further understanding of the forgiveness of poor adherence to treatment and consequently provide guidance for the evaluation of alternative dosing regimens as well as improved recommendations for prescribers and patients. In the previous chapter we have proposed a model-based approach to evaluate forgiveness of drug to treatment interruptions and deviations from the prescribed regimen using an integrated approach in which the pharmacokinetic characteristics and the potency of the drug were integrated with the properties of the viral system in order to predict treatment outcome. Herein, the same concept is applied to a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen and the pharmacokinetic-pharmacodynamic relationships of antiretroviral (ART) drugs are used to evaluate the effect of partial adherence to combination therapy. NNRTI-based regimens are widely administered as combination therapy in children, as recommended by the revised WHO guidelines (3). According to the guidelines, first-line antiretroviral therapy for HIV-infected children older than 3 years of age should include two nucleoside reverse transcriptase inhibitors (NRTI) and a non-nucleoside reverse transcriptase inhibitor. At the moment, the combination between abacavir, lamivudine and efavirenz (ABC-3TC-EFV) is one of the most commonly prescribed to the paediatric population (4). In addition, it has been established that high levels of adherence to NNRTI-based regimens are required in order to achieve and maintain viral suppression (1). However, further investigation is needed to establish in a quantitative and systematic manner what is the clinical relevance of different levels of adherence.

The objective of this study was therefore to evaluate different scenarios of poor adherence in a hypothetical population of 100 HIV-infected children between 3 and 12 years old receiving

abacavir, lamivudine and efavirenz according to the currently recommended doses. Using clinical trial simulations we evaluate relevant scenarios of poor adherence, whilst discriminating the contribution of pharmacokinetic and pharmacodynamic factors from patient behaviour as determinants of variability in treatment response. Moreover, our approach allows scrutiny of the aforementioned factors without subjecting children to the burden of an actual clinical protocol, which in many cases might not be feasible due to ethical reasons. In contrast to the empiricism that has prevailed in the development of antiretroviral therapies, we envisage that our work will shed light on how adherence concepts can be used in conjunction with pharmacokinetic-pharmacodynamic relationships to evaluate the efficacy of drug combinations in HIV-infected children.

9.2. METHODS

Clinical trial simulation

Clinical trial simulations were performed in R (v 2.14.0) using a general template for clinical trial simulation previously developed by our group (5). In figure 1 an overview of the different simulation steps required to implement a clinical trial simulation able to assess the impact of different patterns of non-adherence for a combination of three drugs is presented.

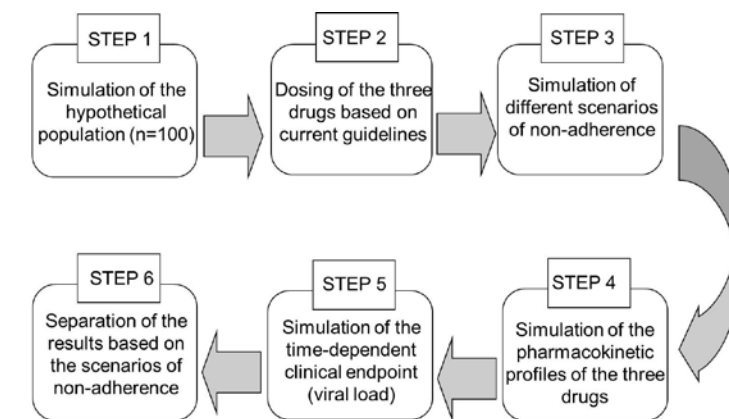


Figure 1 Flow diagram showing the simulation steps required for the implementation of the clinical trial simulations.

Population demographics and treatment

A hypothetical population consisting of 100 HIV-infected between 3 and 12 years old was selected with individuals evenly spread across the body weight range relevant to this group. Body weight has been simulated from an empirical distribution represented by two clinical trials in which HIV-infected children were enrolled, namely PENTA 13 (6) and ARROW part 1 (7)). Simulations of body weight using a multivariate distribution were performed in R (v 2.14.0) according to the method described by Tannenbaum *et al* (8).

Patients in this population were given a combination of three drugs: abacavir (NRTI), lamivudine (NRTI) and efavirenz (NNRTI), which is the currently recommended first-line therapy for HIV-infected children (9). The doses used for each compound were based on the latest Summary of Product Characteristics (table 1). The duration of the hypothetical trial was 48 weeks, with viral load at week 48 as the primary endpoint for evaluation of efficacy. A measure of viral load < 50 copies/mL (detection limit) at week 48 was assumed to be treatment success.

Table 1 Doses of antiretroviral drugs currently recommended for the treatment of HIV-infected children, which were used in the simulation scenarios

Antiretroviral drug	Dosing regimen
Efavirenz	250 mg once daily (14-20 kg) 300 mg once daily (20-25 kg) 350 mg once daily (25-32 kg) 400 mg once daily (32-39 kg) 600 mg once daily (>39 kg)
Lamivudine	150 mg twice daily (14-21 kg) 225 mg twice daily (21-30 kg) 300 mg twice daily (>30 kg)
Abacavir	300 mg twice daily (14-21 kg) 450 mg twice daily (21-30 kg) 600 mg twice daily (>30 kg)

Models for drug pharmacokinetics, drug sensitivity and drug efficacy

Three mathematical models were subsequently used to predict the effect of sub-optimal adherence on treatment outcome. First a pharmacokinetic model was used to predict the time course of plasma concentrations for each drug. Subsequently a pharmacodynamic model was applied to predict the time varying inhibitory effect of the drugs and ultimately a disease model for viral replication and infection was employed to predict the time varying clinical endpoint (viral load).

The pharmacokinetic profiles over time for each drug were simulated based on validated models available in literature (10–12). The median inhibitory concentration (IC₅₀) was used to quantify agent-specific drug susceptibility. To model within-host changes over time in IC₅₀ attributable to the emergence of new-drug resistant mutations the function described in equation (1) was used (13):

$$IC_{50}(t) = \begin{cases} I_0 + \frac{I_r - I_0}{t_r} t & \text{for } 0 < t < t_r \\ I_r & \text{for } t \geq t_r \end{cases} \quad (1)$$

where I_0 and I_r are, respectively, the values of $IC_{50}(t)$ at baseline and time point t_r at which the resistant mutations dominate. If $I_r = I_0$, no new drug resistant mutation is developed during treatment. More complicated models for IC_{50} are available in literature (14, 15), however in clinical practice it is common to collect IC_{50} values only at baseline and failure time (16). The values of IC_{50} for the drugs included in our analysis were obtained from literature, such as the values of I_r , which is based on phenotypic resistance tests for each drug (17–19).

Drug efficacy was considered to be constant over time in several viral dynamic studies (20–22). However, drug efficacy may vary because of the differences in drug concentration and other factors (i.e. development of resistance), which may vary during treatment. A pharmacodynamic sigmoidal additive I_{max} model was used to describe the concentration-effect relationship of three drugs and subsequently predict time-varying efficacy during the course of therapy with the drug combination:

$$\gamma(t) = \frac{\text{ConcEFV}(t)/IC_{50}\text{EFV}(t) + \text{Conc3TC}(t)/IC_{50}3\text{TC}(t) + \text{ConcABC}(t)/IC_{50}\text{ABC}(t)}{\Phi + \text{ConcEFV}(t)/IC_{50}\text{EFV}(t) + \text{Conc3TC}(t)/IC_{50}3\text{TC}(t) + \text{ConcABC}(t)/IC_{50}\text{ABC}(t)} \quad (2)$$

where $\text{ConcEFV}(t)$ is time-varying efavirenz concentration, $\text{Conc3TC}(t)$ is time-varying lamivudine concentration, $\text{ConcABC}(t)$ is time-varying abacavir concentration, $IC_{50}\text{EFV}(t)$ is efavirenz IC_{50} , $IC_{50}3\text{TC}(t)$ is lamivudine IC_{50} and $IC_{50}\text{ABC}(t)$ is abacavir IC_{50} ; Φ is a factor which defines the in vitro-in vivo correlation and it was fixed to 1.

Time varying efficacy $\gamma(t)$ of the treatment ranges from 0 to 1 and is referred to as the treatment efficacy index (the inhibition rate of viral replication) in the viral dynamic model (Equation 3-5). If $\gamma(t) = 1$, the treatment is 100% effective, whereas if $\gamma(t) = 0$, the treatment has no effect.

Mathematical models for HIV dynamics

A widely used model for viral infection and replication was used to simulate the time course of viral load (16). The model is basic and consists of 3 compartments: uninfected target cells (T), free virions (V) and infected target cells (T^*). Although more complex models for viral replication were available in literature, this simple model showed characteristics of stability and robustness which were suitable for the purpose of our analysis.

The model for viral replication is defined by three differential equations (equation 3, 4 and 5). Several parameters are used to describe the viral and immunological processes: λ ($\text{day}^{-1} \text{mm}^{-3}$) is the birth rate of new T cells created in the body, d_T (day^{-1}) is the death rate of the T-cells, N is the amount of virions produced by each of the infected target cells, c (day^{-1}) is the clearance of the free virions, k ($\text{day}^{-1} \text{mm}^{-3}$) is the infection rate, δ (day^{-1}) is the death rate of infected target cells; $\gamma(t)$ is the time varying inhibitory effect of the combination, predicted using the pharmacodynamic model depicted in equation (2).

$$dT / dt = \lambda - d_T - [1 - \gamma(t)] kTV \quad (3)$$

$$dT^* / dt = [1 - \gamma(t)] kTV - \delta T^* \quad (4)$$

$$dV / dt = N\delta T^* - cV \quad (5)$$

Adherence-resistance relationship

A correlation between adherence and resistance in antiretroviral drugs has been previously established by Bangsberg *et al* (23). Despite their initial findings, different correlations appear to exist for different classes of antiretroviral drugs (24). For example, most of the NNRTI and lamivudine mutations occurred in patients showing low adherence, suggesting that these two drugs have different adherence-resistance relationships compared to PIs and NRTIs. In our investigation a relationship between adherence and resistance was considered for efavirenz and lamivudine, given that these drugs are known to be more susceptible to resistance than abacavir (25, 26). Such a relationship was described in terms of a logistic regression (figure 2), in which the probability of resistance is linked to the number of days during which the levels of efavirenz remain below the target therapeutic level or lamivudine remains below the quantification limit. The final model describing the correlation and the corresponding parameter estimates used in the simulations were derived from published data (27, 28).

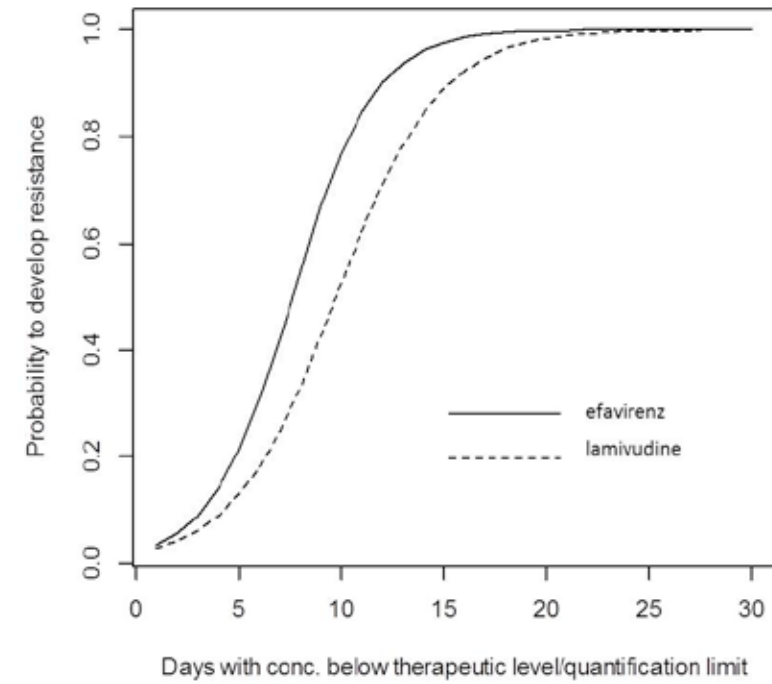


Figure 2 Logistic regression used to include the adherence-resistance relationship for efavirenz and lamivudine. PT50 values were set, respectively, at 8 and 10 days for efavirenz and lamivudine.

Simulation scenarios of variable adherence to treatment

The diagram in figure 3 depicts all the scenarios which were simulated for different patterns of non-adherence to treatment (treatment interruption, delay in drug intake or single doses randomly missed throughout the clinical trial). Based on average adherence in clinical trials derived from objective measures (29–31), a predefined fraction of patients (40%) was randomly selected to be non-adherent to the prescribed dosing regimen. For each scenario the period of non-adherence was randomly assigned to each subject across the duration of the trial. Dropout rates were considered to be zero for the purpose of this analysis. 100 replicates were simulated for each scenario. Simulations were performed with NONMEM v7 and R (v 2.14.0) was used to produce the datasets for each simulation scenario and to numerically and graphically summarise the results. A comparison between treatment outcome for the full population and for the subject non-compliant in case of perfect and imperfect adherence was made for each scenario of non-adherence in order to evaluate the forgiveness of non-adherence of a NNRTI-based regimen in HIV-infected children.

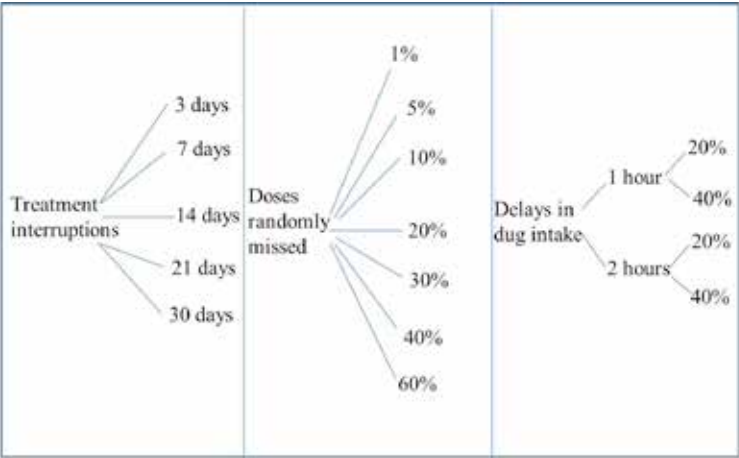


Figure 3 Diagram depicting the simulation scenarios of non-adherence to treatment investigated during the analysis.

9.3. RESULTS

Under assumption of constant resistance rate, our simulations show that viral success is 10% for patients who show perfect adherence. Our simulations reveal that delays in drug intake do not affect treatment outcome of ABC/3TC-EFV combination in HIV-infected children between 3 and 12 years old. As can be seen in figure 4, the proportion of children who achieve viral success at week 48 in case of a delay in drug intake up to 4 hours in 20% or 40% of the days of treatment is not significantly different from the group showing perfect adherence. A closer evaluation of the viral load profiles also shows that delays in drug intake do not affect treatment outcome considering either the full population of 100 HIV-infected children or only the children who were not compliant to the prescribed dosing regimen. On the other hand, interruptions to the NNRTI-based regimen (i.e., simultaneous interruption of the three drugs) seem to have important implications for the outcome of antiretroviral therapy in this population of children. The treatment appears to be forgiving to interruptions of three days, whilst interruptions of one week may decrease the proportion of children experiencing treatment success at the end of the treatment from 90% to 87%, as compared to perfect adherence. A higher effect is observed for treatment interruptions of two weeks, which lead to a decrease the proportion of patients experiencing viral success from 90% to 78% (figure 5). In addition, treatment interruptions of 30 days caused a decrease in the proportion of children achieving viral failure from 90% to 58%.

By contrast, randomly missing 30 doses (i.e., 10% of the prescribed doses during the trial) resulted in a reduction in viral success from 90 to 80% (figure 6).

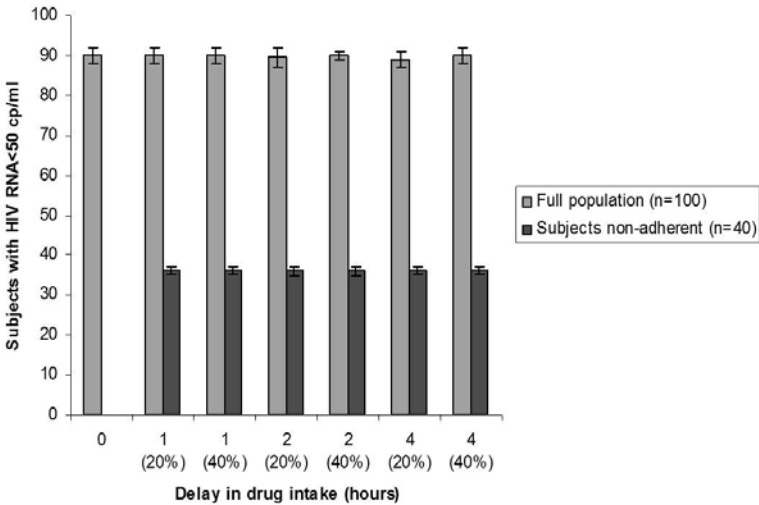


Figure 4 Proportion of subjects achieving viral success (HIV RNA<50 copies/mL) at week 48 based on different delays in drug intake. On the x-axis the number of hours of delay in drug intake is depicted. In parenthesis the percentage of days of treatment in which the delay happens is displayed.

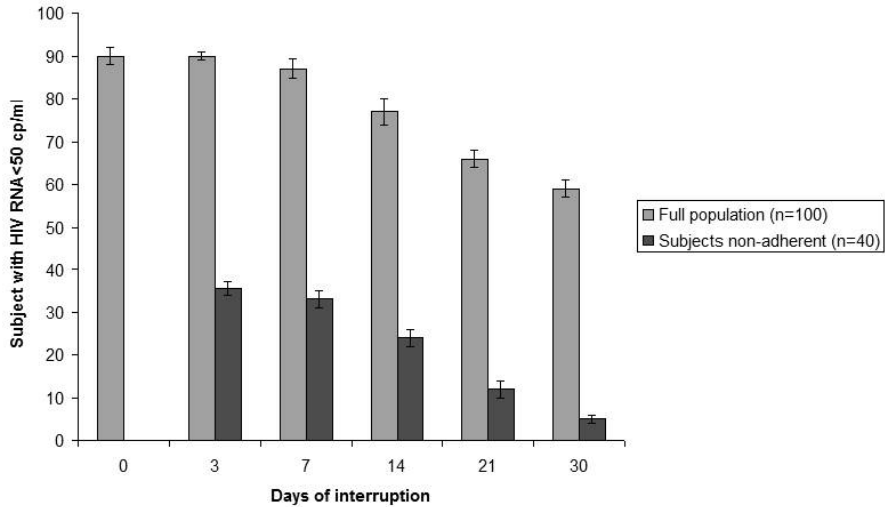


Figure 5 Proportion of subjects achieving viral success at week 48 based on different duration of treatment interruption. On the x-axis the duration (days) of the treatment interruption is depicted.

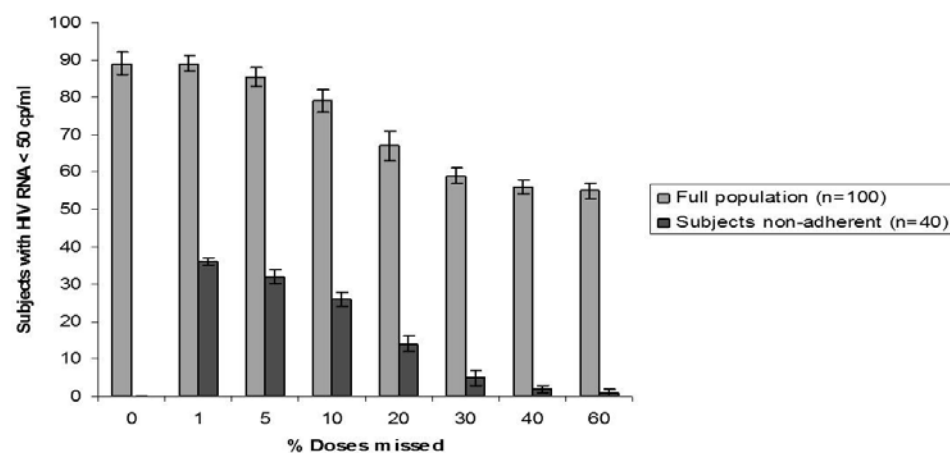


Figure 6 Proportion of subjects achieving viral success at week 48 based on different percentages of doses randomly missed. On the x-axis the percentage of doses missed is displayed.

9.4. DISCUSSIONS AND CONCLUSIONS

This is the first attempt to describe in a quantitative manner the impact of poor adherence to a NNRTI-based regimen when evaluating efficacy in a paediatric population. Given that high levels of adherence in paediatric antiretroviral therapy are very difficult to reach, efforts to identify which conditions represent a higher risk for viral failure is essential for ensuring positive treatment outcome. Using a model-based approach, we have shown that integration of pharmacokinetic and pharmacodynamic models to a disease model for viral replication and infection can provide the basis for a framework for the evaluation of patient behaviour during a clinical trial. In fact, our results are in agreement with a previous investigation (32) which showed that in patients with incomplete adherence, missing doses over a continuous and sustained interval may pose more risk for virologic failure than interspersed missed doses.

The possibility to identify the duration of treatment interruptions that pose more risk for virologic rebound in a specific population and for a specific drug combination represents a powerful tool for mitigation strategies during drug development and risk management after drug approval. In addition, our analysis shows the relevance of clinical trial simulations for the evaluation of experimental design factors that cannot be controlled in a formal manner in real clinical trials. The availability of such an *in silico* methodology offers an alternative to the evaluation of clinically relevant questions in paediatric research, which may not be addressed in real proto-

cols due to ethical and practical issues. Moreover, it enables the characterisation of patterns of non-adherence related to virologic failure before patients are exposed to the treatment. From a clinical safety perspective, this type of information can be transcribed into the label and communicated to prescribers and patients, with potential changes to the benefit-risk balance. It should be noted that the methodology developed here may be easily adapted to different treatment combinations. Furthermore, we can envisage different applications aimed at the evaluation of the robustness of different drug combinations, allowing different treatment options to be compared. It is also conceivable that a different treatment may be prescribed to a patient based on his life-style or adherence records. These opportunities are discussed in the next paragraphs.

Optimisation of antiretroviral therapy

The possibility to simplify antiretroviral therapy is crucial to improve patient adherence and consequently the proportion of patients achieving viral success. In fact, the use of fixed-dose combinations has been a huge success in antiretroviral treatment in adults. However, new optimised dosing regimens are still required given that poor adherence to therapy remains a determinant of resistance. The methodology developed here may be used to test the forgiveness of poor adherence for new simplified dosing regimens, which may not necessarily be higher. In other words, a simplified regimen may improve compliance to treatment, but may not always lead to improved forgiveness in those patients who remain non-adherent. Thus far, this issue has not been evaluated in a systematic manner before enrolling the patients into a clinical trial with a fixed-dose combination.

Pharmacokinetic-pharmacodynamic relationship as a “proxy” for efficacy

Fixed-combination medicinal products have been increasingly used either to improve compliance or to benefit from the added effects of the two or more medicinal products given together. According to the current EMA guideline on fixed-dose combinations (33), the proposed combination should always be based on valid therapeutic principles. In addition, it is necessary to assess the potential advantages (e.g. product rapidly effective, higher efficacy or equal efficacy and better safety) in the clinical situation against possible disadvantages (e.g. cumulative toxicity), for each fixed-combination product and for each dose of the fixed-combination product. In fact, the effectiveness of a simplified dosing regimen has been assessed through the use of pharmacokinetic, efficacy and safety trials (34–36). Given that switching to a simplified dosing regimen may be considered as a particular scenario of forgiveness of non-adherence, we anticipate that the use of this type of *in silico* evaluation will offer the advantage of using pharmacokinetic-pharmacodynamic relationships as a proxy for efficacy, preventing the need for costly and often complex efficacy trials in which it is not the efficacy (and safety) of the drugs or dose levels that are scrutinised, but rather the implications of the alternative dosing regimen. Analogously,

a similar situation exists when pharmacokinetics is used as “proxy” for efficacy in bioequivalence studies.

Potential limitations

The main limitation in our analysis is that toxicity was not taken into account in the simulation scenarios. It is well known that adverse events may influence the level of adherence of the patient. Taking into account the safety profile of abacavir and lamivudine and the mild adverse events that they seem to cause, efavirenz is the only drug for which toxicity should have been a concern. However, no association was found between efavirenz plasma concentrations and the risk of treatment discontinuations because of adverse events or toxicity (37).

Another potential limitation is the fact that we have deliberately chosen to use a basic model of viral dynamics. More complicated models are available that describe viral infection and replication, including different steps and physiological compartments associated with immune response and viral resistance. We do not expect that these models would lead to significantly different results. On the other hand, it is important to point out that in our methodology the inhibitory effect of each single drug could not be evaluated separately. Rather, it was expressed through an additive equation. Although in vitro studies may be used to evaluate the inhibitory effect of a single drug, assessment of the contribution of each drug to the total inhibitory effect of a combination are usually not performed. Here we show that despite the lack of such information, the use of a model-based approach enables the evaluation of the inhibitory effects in vivo of three drugs administered in combination.

Conclusions

In summary, we have shown that for similar levels of compliance treatment interruptions may pose a higher risk for viral rebound, as compared to randomly missed doses when using NNR-TI-based regimens. Our analysis also illustrates how mathematical and statistical models can be used to investigate adherence scenarios which cannot be evaluated in clinical practice in paediatric HIV due to obvious ethical and practical reasons. This methodology offers the opportunity for the development of patient-tailored treatments taking into account differences in compliance pattern. Further investigation of the impact of non-adherence to different antiretroviral combination treatments (e.g. boosted protease inhibitor-based regimens) is required to confirm the generalisability of the approach used here.

References

1. Bangsberg, D.R. *et al.* Non-adherence to highly active antiretroviral therapy predicts progression to AIDS. *AIDS (London, England)* **15**, 1181-3 (2001).
2. Genberg, B.L. *et al.* Patterns of antiretroviral therapy adherence and impact on HIV RNA among patients in North America. *AIDS (London, England)* **26**, 1415-1423 (2012).
3. Antiretroviral therapy for HIV infection in infants and children. Available at: <http://www.who.int/hiv/pub/paediatric/paed-prelim-summary.pdf> (last accessed 20 June 2013)
4. Elzi, L. *et al.* Choice of initial combination antiretroviral therapy in individuals with HIV infection: Determinants and Outcomes. *Archives of internal medicine* **172**, 1313-1321 (2012)
5. Teutonico, D. *et al.* Development of a template for clinical trial simulations in COPD. *Poster presented at PAGE Conference 2010*
6. Bergshoeff, A. *et al.* Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
7. Musiime, V. *et al.* Pharmacokinetics and acceptability of once- versus twice-daily lamivudine and abacavir in HIV type-1-infected Ugandan children in the ARROW Trial. *Antiviral therapy* **15**, 1115-24 (2010).
8. Tannenbaum, S.J., Holford, N.H.G., Lee, H., Peck, C.C. & Mould, D.R. Simulation of correlated continuous and categorical variables using a single multivariate distribution. *Journal of pharmacokinetics and pharmacodynamics* **33**, 773-94 (2006).
9. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. Available at: <http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf> (last accessed 20 June 2013)
10. Dickinson, L. *et al.* Sequential population pharmacokinetic modeling of lopinavir and ritonavir in healthy volunteers and assessment of different dosing strategies. *Antimicrobial agents and chemotherapy* **55**, 2775-82 (2011).
11. Kappelhoff, B.S. *et al.* Population pharmacokinetics of efavirenz in an unselected cohort of HIV-1-infected individuals. *Clinical pharmacokinetics* **44**, 849-61 (2005).

12. Zhao, W., Piana, C., Danhof, M., Burger, D., Pasqua, O.D. & Jacqz-Aigrain, E. Population pharmacokinetics of abacavir in infants, toddlers and children. *British journal of clinical pharmacology* **75**(6),1525-35 (2013)
13. Huang, Y., Rosenkranz, S.L. & Wu, H. Modeling HIV dynamics and antiviral response with consideration of time-varying drug exposures, adherence and phenotypic sensitivity. *Mathematical biosciences* **184**, 165-86 (2003).
14. Bonhoeffer, S., Lipsitch, M. & Levin, B.R. Evaluating treatment protocols to prevent antibiotic resistance. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12106-11 (1997).
15. Wainberg, M.A., Hsu, M., Gu, Z., Borkow, G. & Parniak, M.A. Effectiveness of 3TC in HIV clinical trials may be due in part to the M184V substitution in 3TC-resistant HIV-1 reverse transcriptase. *AIDS (London, England)* **10 Suppl 5**, S3-10 (1996).
16. Wu, H. *et al.* Modeling long-term HIV dynamics and antiretroviral response: effects of drug potency, pharmacokinetics, adherence, and drug resistance. *Journal of acquired immune deficiency syndromes (1999)* **39**, 272-83 (2005).
17. Fletcher, C.V. *et al.* Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. *AIDS (London, England)* **14**, 2137-44 (2000).
18. Melroy, J. & Nair, V. The antiviral activity, mechanism of action, clinical significance and resistance of abacavir in the treatment of pediatric AIDS. *Current pharmaceutical design* **11**, 3847-52 (2005).
19. Best, B.M. *et al.* Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. *The Journal of antimicrobial chemotherapy* **66**, 354-7 (2011).
20. Wein, L.M., D'Amato, R.M. & Perelson, A.S. Mathematical analysis of antiretroviral therapy aimed at HIV-1 eradication or maintenance of low viral loads. *Journal of theoretical biology* **192**, 81-98 (1998).
21. Wu, H. & Ding, A.A. Population HIV-1 dynamics in vivo: applicable models and inferential tools for virological data from AIDS clinical trials. *Biometrics* **55**, 410-8 (1999).
22. Ding, A.A. & Wu, H. Relationships between antiviral treatment effects and biphasic viral decay rates in modeling HIV dynamics. *Mathematical biosciences* **160**, 63-82 (1999).
23. Bangsberg, D.R., Kroetz, D.L. & Deeks, S.G. Adherence-resistance relationships to combination HIV antiretroviral therapy. *Current HIV/AIDS reports* **4**, 65-72 (2007).
24. Tam, L.W.Y. *et al.* The Relationship Between Resistance and Adherence in Drug-Naive Individuals Initiating HAART Is Specific to Individual Drug Classes. *British Columbia Centre for Excellence* **49**, 266-271 (2008).
25. Bachelier, L. *et al.* Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. *Journal of virology* **75**, 4999-5008 (2001).
26. Frost, S.D., Nijhuis, M., Schuurman, R., Boucher, C.A. & Brown, A.J. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. *Journal of virology* **74**, 6262-8 (2000).
27. Ribaud, H.J. *et al.* Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **42**, 401-7 (2006).
28. Bachelier, L.T. *et al.* Human immunodeficiency virus type 1 mutations selected in patients failing efavirenz combination therapy. *Antimicrobial agents and chemotherapy* **44**, 2475-84 (2000).
29. Arnsten, J.H. *et al.* Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **33**, 1417-23 (2001).
30. Gross, R., Bilker, W.B., Friedman, H.M. & Strom, B.L. Effect of adherence to newly initiated antiretroviral therapy on plasma viral load. *AIDS (London, England)* **15**, 2109-17 (2001).
31. Liu, H. *et al.* A comparison study of multiple measures of adherence to HIV protease inhibitors. *Annals of internal medicine* **134**, 968-77 (2001).
32. Parienti, J.-J. *et al.* Not all missed doses are the same: sustained NNRTI treatment interruptions predict HIV rebound at low-to-moderate adherence levels. *PloS one* **3**, e2783 (2008).
33. European Medicines Agency. Guideline on Fixed Combination Medicinal Products. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003689.pdf (last accessed 20 June 2013)

34. Chokephaibulkit, K. *et al.* Once- versus twice-daily lopinavir/ritonavir tablets in virologically suppressed, HIV-infected, treatment-experienced children: comparative pharmacokinetics and virological outcome after switching to once-daily lopinavir/ritonavir. *The Journal of antimicrobial chemotherapy* **67**, 2927-31 (2012).
35. Nacro, B. *et al.* Pharmacology and immuno-virologic efficacy of once-a-day HAART in African HIV-infected children: ANRS 12103 phase II trial. *Bulletin of the World Health Organization* **89**, 451-8 (2011).
36. McKinney, R.E. *et al.* Long-term safety and efficacy of a once-daily regimen of emtricitabine, didanosine, and efavirenz in HIV-infected, therapy-naïve children and adolescents: Pediatric AIDS Clinical Trials Group Protocol P1021. *Pediatrics* **120**, e416-23 (2007).
37. van Luin, M. *et al.* Absence of a relation between efavirenz plasma concentrations and toxicity-driven efavirenz discontinuations in the EuroSIDA study. *Antiviral therapy* **14**, 75-83 (2009).

FORGIVENESS OF NON-ADHERENCE TO ONCE VS. TWICE DAILY LAMIVUDINE/ABACAVIR IN COMBINATION WITH EFAVIRENZ IN HIV-INFECTED CHILDREN

Chiara Piana, Donato Teutonico, Meindert Danhof, Oscar Della Pasqua

SUMMARY

Optimisation of therapeutic management of infectious diseases is a priority in the European Union. Harmonisation of dosing regimens in order to encourage the development of fixed-dose combinations is therefore encouraged. Fixed-dose combinations are important as they can improve how well a patient is able to follow medical advice in terms of taking medicine at the right time and taking the correct number and combination of tablets. However, one of the main concerns of once daily dosing regimens based on fixed-dose combinations is the potentially lower robustness compared to twice daily dosing. This study was aimed at evaluating the forgiveness of non-adherence to once vs. twice daily lamivudine/abacavir.

Clinical trial simulations (CTS) were performed in R 2.14. Once and twice daily lamivudine/abacavir were administered with efavirenz to a hypothetical population of HIV-infected children between 3 and 12 years old ($n=30$). Different degrees of adherence to therapy were simulated for the two regimens. The primary efficacy endpoint was the measure of viral load at week 48.

Our results show comparable forgiveness of non-adherence to the two dosing regimens in case of treatment interruptions, doses randomly missed and delays in drug intake.

Based on these findings, it appears that once daily lamivudine/abacavir could be administered in combination with efavirenz to children between 3 and 12 years old without any risk of decreased efficacy, as compared to the currently recommended twice daily regimen.

10.1. INTRODUCTION

Several attempts have been made to improve patient adherence to antiretroviral therapy. Among other things, the development of fixed-dose combinations and the administration of once daily dosing options have determined a significant reduction in pill burden (1). Currently once daily dosing regimens seem to be the preferred treatment options to improve patient adherence (2). In this context, a once daily single-tablet fixed-dose combination represents an important simplification of HIV treatment. Fixed-dose combinations include drugs with favourable pharmacokinetics that do not need dose adjustments, have no additional toxicities, and do not require dissimilar intake conditions (3). In fact, it has been shown that in newly diagnosed antiretroviral-naïve patients, once daily dosing of antiretroviral drugs resulted in higher adherence than twice daily dosing (4).

Regardless of such advantages, one of the main regulatory concerns with modifications in a dosing regimen is that treatment response may change or be less robust as compared to the reference dosing regimens. In other words, despite the endorsement of current guidelines as to the importance of characterising the relationships between dose, drug concentrations and clinical response for the safe and effective use of drugs in individual patients, including the possibility to define dosage and administration instructions in the product label (5), confirmatory clinical trials are still deemed necessary to prove efficacy of new regimens for fixed-drug combinations, preferably by parallel group comparisons in which the new regimen is compared to the reference treatment (6). In most cases, these requirements appear to contradict each other, in that knowledge of pharmacokinetic-pharmacodynamic relationships should enable accurate inferences of efficacy and safety. One should therefore dispute the need for confirmatory trials. Scientifically, what should be evaluated is whether the underlying pharmacokinetic-pharmacodynamic relationships are not affected by changes in dosing regimen.

In reality, this concern pertains to the realm of quantitative clinical pharmacology and is closely related to the concept of compliance, treatment adherence and forgiveness. In conjunction with models of disease dynamics, pharmacokinetic-pharmacodynamic relationships can be used to explore whether simplified dosing regimens yield different response to therapy as compared to more frequent dosing regimens.

In chapter 9 we have shown that a model-based approach may be used to assess the effect of partial adherence to a fixed-drug combination of abacavir, lamivudine and efavirenz. Our results suggest that pharmacokinetic-pharmacodynamic relationships of antiretroviral drugs could be used as “proxy” of efficacy. Moreover, it shows that the evaluation of forgiveness of non-adherence for simplified dosing regimens is crucial to assess the feasibility of alternative treatment options.

In contrast to current guidelines, which impose pharmacokinetic, efficacy and safety trials to assess the suitability of a simplified dosing regimen, this study aims at showing how pharmaco-

kinetic-pharmacodynamic relationships may be used as predictors of response to changes in dosing regimen, thereby avoiding the need to unnecessarily enroll children into an efficacy trial.

Here we use abacavir and lamivudine as a paradigm combination to explore the forgiveness of treatment to changes in dosing regimen. The use of dual nucleoside reverse transcriptase inhibitors (NRTI) is recommended as a part of combination antiretroviral therapy (7). Abacavir (ABC) and lamivudine (3TC) constitute the NRTI backbone in HIV-therapy and are usually administered in adults and children in combination with one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one boosted protease inhibitor (PI). At present abacavir and lamivudine are administered as twice daily in children and once daily in adults. Among the various trials in which abacavir and lamivudine have been investigated in children, the PENTA 13 trial demonstrated similar pharmacokinetics between once and twice daily doses, after administration of either a liquid or tablet formulation (8). In the same study, acceptability data demonstrated that caregivers preferred once daily dosing only if all drugs in the regimen were once daily. The PENTA 15 trial also demonstrated similar pharmacokinetics between once and twice daily dosing with abacavir and lamivudine in children aged 3 months- 3 years old (9). In addition, a sub-study of the ARROW trial (a randomised trial monitoring practice and induction maintenance of antiretroviral therapy regimens) (10) has shown that once daily regimens resulted in equivalent AUC_{0-24} to twice daily regimens in 3 to 12 year-old African children receiving World Health Organization (WHO) recommended doses of scored tablets. It should also be noted that two previous population pharmacokinetic analyses have been performed by our group, which showed comparable exposure of once and twice daily lamivudine and abacavir in a large simulation of a paediatric population including children between 3 months and 12 years old.

Based on the points discussed above, it is clear that the evaluation of once daily dosing of lamivudine and abacavir in children will be crucial for treatment optimisation and further alignment with its current use in the adult indication (11). Despite similar pharmacokinetic exposure for the two dosing regimens, the impact of non-adherence on treatment outcome should be investigated to ensure the robustness of once daily lamivudine/abacavir.

10.2. METHODS

Clinical trial simulation

The simulation scenarios described in the next paragraphs were performed in R (v 2.14.0) using a clinical trial simulations template, which was developed by our group (12). In figure 1 an overview is presented of the different simulation steps required to implement a clinical trial simulation aimed at the assessment of the impact of non-adherence for a combination of three drugs after once and twice daily dosing regimens.

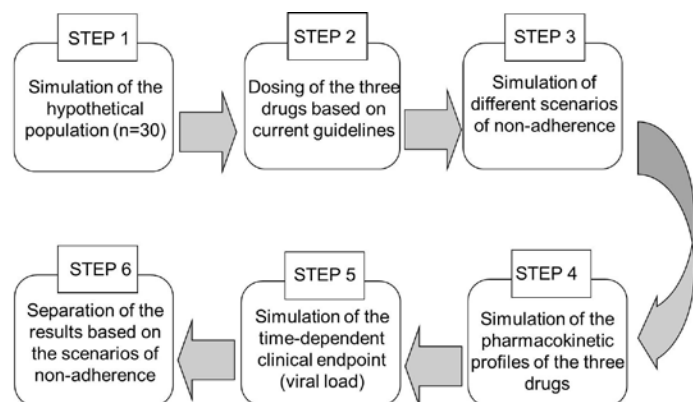


Figure 1 Diagram depicts the simulation steps required to implement the clinical trial simulation.

Population demographics and treatment

A hypothetical population consisting of 200 HIV-infected between 3 and 12 years old was selected with individuals evenly spread across the body weight range relevant to this group. Body weight has been simulated from an empirical distribution represented by two clinical trials in which HIV-infected children were enrolled, namely PENTA 13 (8) and ARROW part 1 (10)). Simulations of body weight using a multivariate distribution were performed in R (v 2.14.0) according to the method described by Tannenbaum *et al* (13). The outcome of the treatment was split into two cases, namely perfect and imperfect adherence. Given the high number of scenarios evaluated and the computational time required, a smaller population was simulated ($n=30$) during the evaluation of the scenarios of poor adherence. However, more subjects ($n=200$) were evaluated when the sample size was deemed important for the interpretation of the results.

Three drugs (abacavir, lamivudine and efavirenz), currently used as first-line therapy in HIV-infected children (14), were administered according to body weight as established in the latest summary of product characteristics. The duration of the hypothetical trial was 48 weeks. The children received the drugs either as twice daily or once daily dosing regimens. The endpoint for efficacy was the probability of viral failure (HIV RNA higher than detection limit) at week 48. Viral failure was defined as a value of viral load higher than the detection limit. The thresholds used as detection limit were 50 and 400 copies/mL in case of perfect adherence. In the subsequent evaluation of the scenarios of poor adherence the strictest threshold of 50 copies/mL was used.

Models for drug pharmacokinetics, drug resistance and drug efficacy

Three mathematical models were subsequently used to predict the effect of sub-optimal adherence on treatment outcome after the use of once or twice daily doses of a fixed-dose combination. First a pharmacokinetic model was used to predict the time course of plasma concentrations for each drug. Subsequently a pharmacodynamic model was applied to predict the time varying inhibitory effect of the drugs and ultimately a disease model for viral replication and infection was employed to predict the time varying clinical endpoint (viral load).

The pharmacokinetic profiles over time for each drug were simulated based on validated models available in literature (15–17). The median inhibitory concentration (IC_{50}) was used to quantify agent-specific drug susceptibility. To model within-host changes over time in IC_{50} attributable to the emergence of new-drug resistant mutations the function described in equation (1) was used (18):

$$IC_{50}(t) = \begin{cases} I_0 + \frac{I_r - I_0}{t_r} t & \text{for } 0 < t < t_r \\ I_r & \text{for } t \geq t_r \end{cases} \quad (1)$$

where I_0 and I_r are, respectively, the values of $IC_{50}(t)$ at baseline and time point t_r at which the resistant mutations dominate. If $I_r = I_0$, no new drug resistant mutation is developed during treatment. More complicated models for IC_{50} are available in literature (19, 20), however in clinical practice it is common to collect IC_{50} values only at baseline and failure time (21). The values of IC_{50} for the drugs included in our analysis were obtained from literature, such as the values of I_r , which is based on phenotypic resistance tests for each drug (22–24).

Drug efficacy was considered to be constant over time in several viral dynamic studies (25–27). However, drug efficacy may vary because of the differences in drug concentration and other factors (i.e. development of resistance), which may vary during treatment. A pharmacodynamic additive sigmoid I_{max} model was used to describe the concentration-effect relationship of three drugs and subsequently predict time-varying efficacy during the course of therapy with the drug combination:

$$v(t) = \frac{ConcEFV(t)/IC_{50}EFV(t) + Conc3TC(t)/IC_{50}3TC(t) + ConcABC(t)/IC_{50}ABC(t)}{\Phi + ConcEFV(t)/IC_{50}EFV(t) + Conc3TC(t)/IC_{50}3TC(t) + ConcABC(t)/IC_{50}ABC(t)} \quad (2)$$

where $ConcEFV(t)$ is time-varying efavirenz concentration, $Conc3TC(t)$ is time-varying lamivudine concentration, $ConcABC(t)$ is time-varying abacavir concentration, $IC_{50}EFV(t)$ is efavirenz IC_{50} , $IC_{50}3TC(t)$ is lamivudine IC_{50} and $IC_{50}ABC(t)$ is abacavir IC_{50} ; Φ is a factor which defines the *in vitro-in vivo* correlation. In our simulations, Φ was fixed to 1.

Time varying efficacy $\gamma(t)$ of the treatment ranges from 0 to 1 and is referred to as the treatment efficacy index (the inhibition rate of viral replication) in the viral dynamic model (Equation 3-5). If $\gamma(t) = 1$, the treatment is 100% effective, whereas if $\gamma(t) = 0$, the treatment has no effect.

Mathematical models for HIV dynamics

A widely used model for viral infection and replication was used to simulate the time course of the clinical endpoint (viral load) (21). The model consists of 3 compartments: uninfected target cells (T), free virions (V) and infected target cells (T^*). Although more complex models for viral replication were available in literature, this simple model showed characteristics of stability and robustness which were suitable for the purpose of our analysis.

The model for viral replication is defined by three differential equations (equation 3, 4 and 5). Several parameters are used to describe the viral and immunological processes: λ ($\text{day}^{-1} \text{mm}^{-3}$) is the birth rate of new T cells created in the body, d_T (day^{-1}) is the death rate of the T-cells, N is the amount of virions produced by each of the infected target cells, c (day^{-1}) is the clearance of the free virions, k ($\text{day}^{-1} \text{mm}^3$) is the infection rate, δ (day^{-1}) is the death rate of infected target cells; $\gamma(t)$ is the time varying inhibitory effect of the combination, predicted using the pharmacodynamic model depicted in equation (2).

$$dT / dt = \lambda - d_T - [1 - \gamma(t)] kTV \quad (3)$$

$$dT^* / dt = [1 - \gamma(t)] kTV - \delta T^* \quad (4)$$

$$dV / dt = N\delta T^* - cV \quad (5)$$

Correlation between adherence and resistance

A correlation between adherence and resistance in antiretroviral drugs has been previously established by Bangsberg *et al* (28). Despite their initial findings, different correlations appear to exist for different classes of antiretroviral drugs (29). For example, most of the NNRTI and lamivudine mutations occurred in patients showing low adherence, suggesting that these two drugs have different adherence–resistance relationships compared to PIs and NRTIs. In our investigation a relationship between adherence and resistance was considered for efavirenz and lamivudine, given that these drugs are known to be more susceptible to resistance than abacavir (30, 31). Such a relationship was described in terms of a logistic regression, in which the probability of resistance is linked to the number of days during which the levels of efavirenz remain below the target therapeutic level or lamivudine remains below the quantification limit. The final model describing the correlation and the corresponding parameter estimates used in the simulations were derived from published data (32, 33).

Simulation scenarios of variable adherence to treatment

Scenarios representing perfect adherence and different patterns of non-adherence to treatment (treatment interruption, delay in drug intake or single doses randomly missed throughout the clinical trial) were simulated. In each scenario of non-adherence all the patients participating in the trial were assumed to be non-adherent to treatment. For each scenario the period of non-adherence was randomly assigned to each subject across the duration of the trial. 100 replicates were simulated for each scenario. Simulations were performed with NONMEM v7 and R v 2.14.0 was used to produce the datasets for each simulation scenario and to numerically and graphically summarise the results. The impact of changes in dosing regimen was assessed by comparing the results obtained in each scenario of non-adherence with that of perfect adherence. A measure of viral load above the threshold of 50 copies/mL was selected to indicate viral failure at week 48.

The results were compared for twice daily and once daily dosing regimens. In order to obtain a quantitative relationship between the probability of viral failure and the percentages of doses randomly missed or the duration of treatment interruptions for each dosing regimen, the data were fitted using the -nls (nonlinear least squares) function in R.

10.3. RESULTS

During our analysis the forgiveness of non-adherence of once daily lamivudine/abacavir has been evaluated and compared to the forgiveness of the currently licensed twice daily dosing in children, both administered in combination with efavirenz as a fixed-dose combination. Initially, the two combination treatments were compared in terms of efficacy assuming perfect adherence to therapy. As shown in figure 2, the probability to experience viral failure at week 48 is not significantly different after once or twice daily doses. In addition, our results show that time-varying viral load remains indistinguishable during the whole trial period, i.e., 48 week. Moreover, the therapeutic equivalence of the treatment is observed at week 48 considering both the detection limits of 50 and 400 copies/mL.

Under the assumption of imperfect adherence, similar findings were observed. As shown in figures 3, 4 and 5, the probability of viral failure at week 48 for the two treatment combinations in a group of 30 HIV infected children does not reveal any significant differences between dosing regimens. The similarities were observed irrespective of whether non-compliance was due to delays in drug intake (figure 3), treatment interruptions (figure 4) or randomly missed doses (figure 5). As expected, delays in drug intake up to 4 hours were shown not to affect the outcome of the treatment (figure 3), whilst changes in the probability of viral failure could be observed for the scenarios depicting treatment interruptions or doses randomly missed. These differenc-

es were independent of the dosing regimen.

To ensure accuracy of the estimated effects, additional simulations were performed for a larger population (n=200), including the most significant scenarios of non-adherence, namely, 4 hours delay in drug intake, two weeks interruptions and 10% of the doses randomly missed. No differences were observed in the results compared to the same scenarios evaluated with 30 patients.

In figure 4 and 5 the quantitative relationships between the probability of viral failure and the duration of treatment interruptions and the percentages of doses randomly missed are shown, respectively. The fitting of the data, illustrated in figure 6, allowed us to derive the mathematical function that links the number of days of treatment interruption with the probability to experience viral failure. Practically, the same parameter estimates were obtained for both once daily and twice daily dosing regimens. As shown in figure 6, a function linking the percentage of doses randomly missed with the probability of viral failure was also obtained for the two regimens.

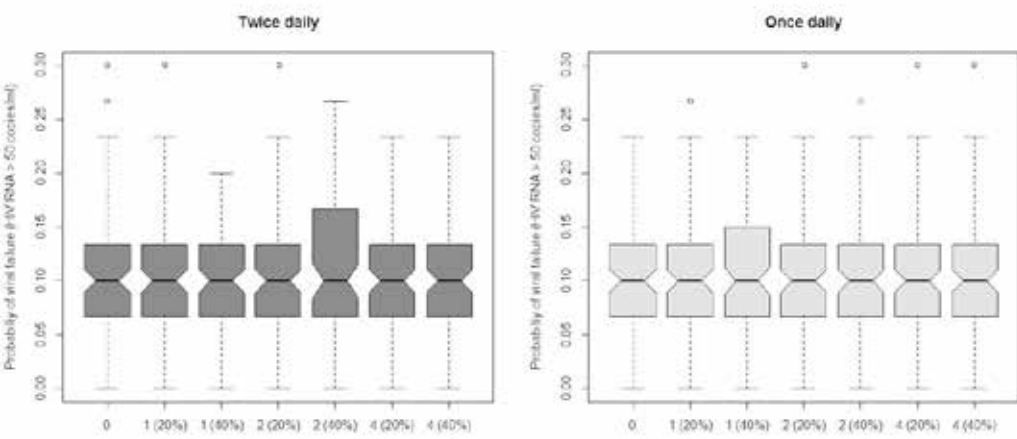


Figure 3 Probability of viral failure associated with variable delay in drug intake for twice daily (left) and once daily (right) dosing regimen. On the x-axis the hours of delay in drug intake are displayed. In parenthesis the percentage of days in which the delay takes place is represented.

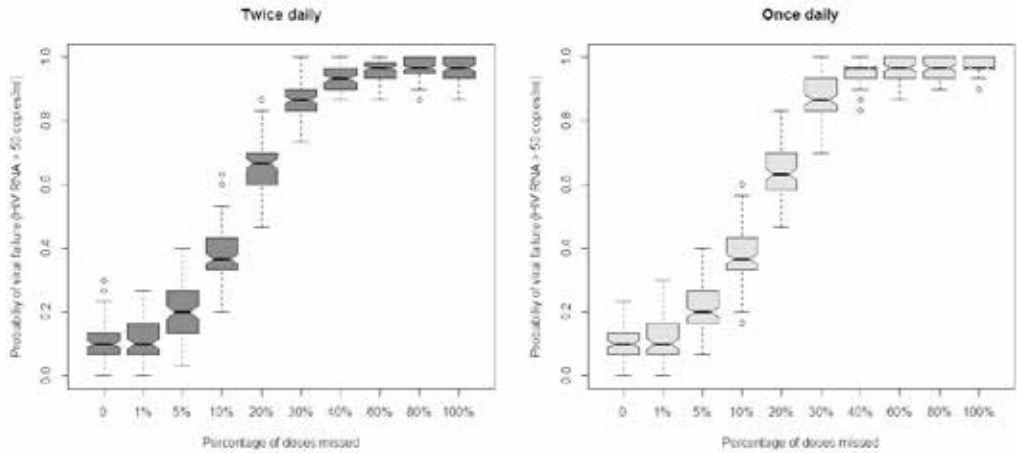


Figure 4 Probability of viral failure associated with variable duration of treatment interruption for twice daily (left) and once daily (right) dosing regimen. On the x-axis the duration of the treatment interruption is displayed.

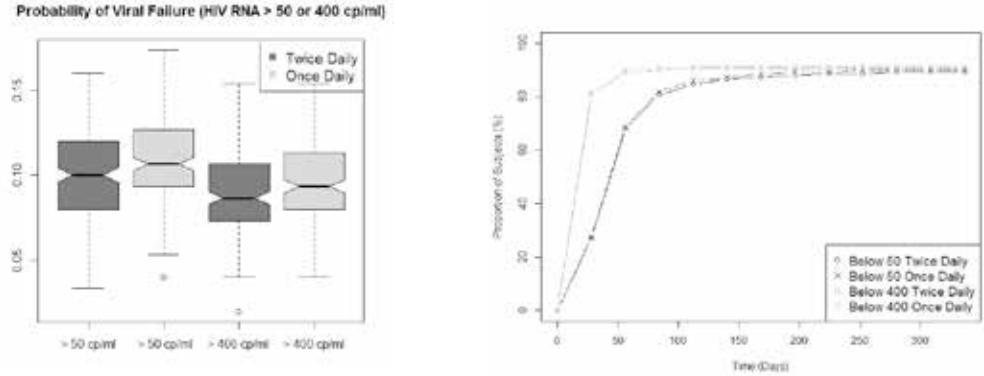


Figure 2 Probability of viral failure in case of perfect adherence for once and twice daily dosing regimen at the end of the treatment (left panel) and across the whole duration of the treatment (right panel).

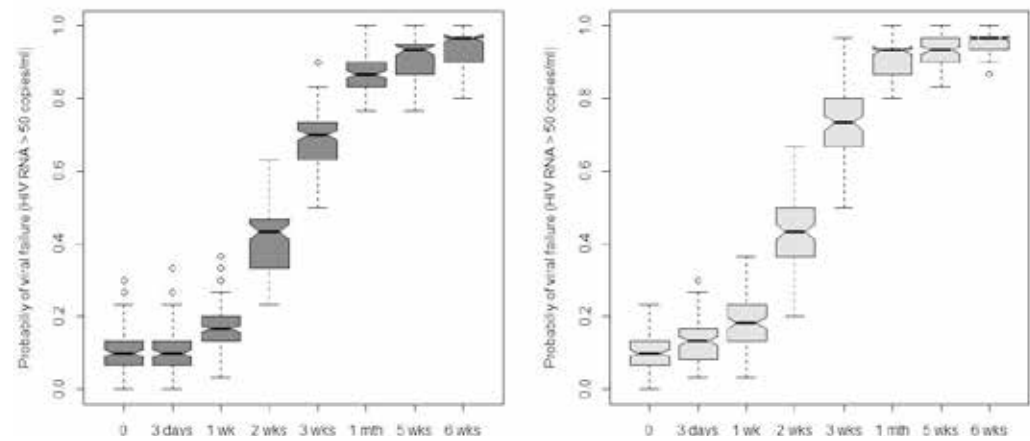


Figure 5 Probability of viral failure associated with variable percentage of randomly missed doses for twice daily (left) and once daily (right) dosing regimen. On the x-axis the percentage of doses missed is displayed.

10.4. DISCUSSIONS AND CONCLUSIONS

Efficacy of once vs. twice daily lamivudine/abacavir in children

This investigation shows the robustness of a simplified treatment combination (efavirenz + lamivudine/abacavir once daily) in terms of its impact on treatment outcome as compared to the currently licensed regimen (efavirenz + lamivudine/abacavir twice daily), taking different patterns of non-adherence into account.

Conceptually, our analysis also demonstrates the relevance of pharmacokinetic-pharmacodynamic relationships as a proxy for the assessment of efficacy when changes are made to dosing regimens. Initially, a comparison of the two treatments was made assuming perfect adherence in order to test whether the dosing regimens would show the same efficacy in ideal conditions. The results summarised in figure 2 clearly shows comparable efficacy for both regimens and corroborate our previous evaluation of the pharmacokinetics of abacavir and lamivudine after oral administration performed in chapters 3 and 4. Besides being used as baseline to evaluate the forgiveness of non-adherence of once daily treatment, these findings reveal for the first time that the delivery of the same drug amount over 24 hours, as assessed in terms of systemic exposure (AUC), correlates with efficacy in children. In fact, such results are in agreement with Sosa *et al.*, who have already shown non-inferiority of once daily fixed-dose combination abacavir/lamivudine compared to twice daily in adults (34).

Optimisation of antiretroviral therapy

In addition to the conceptual aspects of the methodology and the evidence of therapeutic equivalence of the two regimens, it is interesting to highlight that our approach allowed the identification of the level of adherence that is required or desirable to prevent the risk of viral rebound for each of the dosing regimens investigated here. The fitting of the data using a logistic model enabled us to obtain mathematical functions linking the number of days of treatment interruption and the percentage of doses randomly missed with the probability of viral failure for the two dosing regimens. Regarding treatment interruptions, our results show that an interruption of two weeks may have consequences for the clinical outcome and may increase the probability of viral failure from 0.1 to above 0.4, whilst a continuous interruption of 30 days yields a probability of viral failure of 0.9. On the other hand, randomly missing as little as 10% of the doses during a treatment of 48 weeks may increase the probability of viral failure from 0.1 up to 0.4. In practical terms, this means that continuous treatment interruptions with a NN-RTI-based regimen are more likely to result in viral failure than when the same number of doses is randomly missed. These figures are in agreement with the investigation of Parienti *et al.* (35), who found that sustained treatment interruptions more closely predicted viral rebound than interspersed missed doses in 72 patients who were administered a NNRTI-based regimen. In their investigation a logistic regression model was used to estimate the relationship between treatment interruptions and probability of viral failure, with treatment interruptions of 15 days associated with a 50% probability of viral rebound. Our results also appear to be in agreement with Cohen *et al.* (36) and Dybul *et al.* (37), who showed that virologic rebound is uncommon among NNRTI treated patients with repeated short cycles on and off treatment.

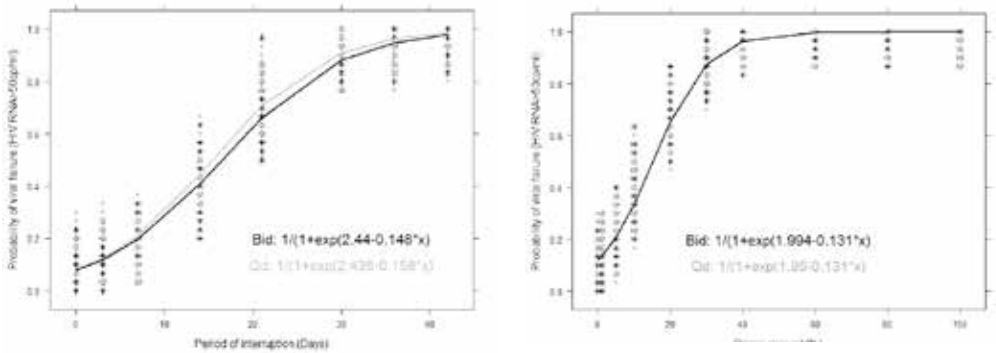


Figure 6 Fitting of the data of probability of viral failure associated with treatment interruption (figure 6a) and doses randomly missed (figure 6b). The fitting was obtained using the nls (Nonlinear least squares) function in R.

Use of clinical trial simulation

In addition to the findings which are specific to the fixed combination of abacavir, lamivudine and efavirenz, our results unravel an important aspect of model-based drug development. The use of models of disease dynamics in conjunction with models describing pharmacokinetic-pharmacodynamic relationships and differences in patterns of drug intake enables the assessment of the predictive value of pharmacokinetic-pharmacodynamic relationships. The ability to make inferences about treatment outcome using such data may avoid the need to expose children to the burden of clinical trial protocols. Based on our investigation, pharmacokinetic-pharmacodynamic studies, combined with pharmacokinetic and safety trials, may suffice for generating evidence of the suitability of a simplified dosing regimen. Furthermore, we show that clinical trial simulations may be applied to generate information which cannot be obtained in clinical practice. Despite improvement in clinical research, it is still impossible to control or explore all the scenarios of non-adherence investigated here. Clinical trial simulations provide a solid basis for study design optimisation before subjecting patients to a clinical protocol.

We are aware that our investigation relies on a hypothetical population of children, which cannot be replicated with real data, as the scenarios evaluated here cannot be easily generated in a clinical protocol. Nevertheless, we are confident in the results given the concordance with the findings observed by Parienti *et al.*, which were based on real data. Formal validation would require a trial with a pre-defined set of scenarios, in which adherence varies as per protocol, rather than randomly.

Conclusions

In summary, we have shown that pharmacokinetic-pharmacodynamic relationships can be used as a proxy for efficacy and that a simplified once daily lamivudine/abacavir regimen administered in combination with efavirenz yields comparable efficacy as the currently licensed twice daily dosing in HIV-infected children between 3 and 12 years old. Moreover, our results show that both regimens show similar forgiveness of poor adherence, which implies exchangeability of the treatment options. These findings represent another step towards the availability of once daily regimens for fixed-dose combinations in children, which are crucial for increased patient adherence to HIV therapy, with direct implications for quality of life of the patients and of their caregivers.

References

1. Ruane, P. *et al.* Pilot study of once-daily simplification therapy with abacavir/lamivudine/zidovudine and efavirenz for treatment of HIV-1 infection. *HIV clinical trials* **7**, 229-36
2. Permpalung, N., Putcharoen, O., Avihingsanon, A. & Ruxrungtham, K. Treatment of HIV infection with once-daily regimens. *Expert opinion on pharmacotherapy* **13**, 2301-17 (2012).
3. Llibre, J.M. & Clotet, B. Once-daily single-tablet regimens: a long and winding road to excellence in antiretroviral treatment. *AIDS reviews* **14**, 168-78 (2012).
4. Buscher, A., Hartman, C., Kallen, M.A. & Giordano, T.P. Impact of antiretroviral dosing frequency and pill burden on adherence among newly diagnosed, antiretroviral-naïve HIV patients. *International journal of STD & AIDS* **23**, 351-5 (2012).
5. European Medicines Agency. Dose Response Information to Support Drug Registration (2006). Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002834.pdf (last accessed 7 June 2013)
6. European Medicines Agency. Guideline on Fixed Combination Medicinal Products. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003689.pdf (last accessed 7 June 2013)
7. Chowers, M., Gottesman, B.-S., Leibovici, L., Schapiro, J.M. & Paul, M. Nucleoside reverse transcriptase inhibitors in combination therapy for HIV patients: systematic review and meta-analysis. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* **29**, 779-86 (2010).
8. Bergshoeff, A. *et al.* Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13). *Antiviral therapy* **10**, 239-46 (2005).
9. Paediatric European Network for Treatment of AIDS (PENTA). Pharmacokinetic study of once-daily versus twice-daily abacavir and lamivudine in HIV type-1-infected children aged 3-<36 months. *Antiviral therapy* **15**, 297-305 (2010).
10. Musiime, V. *et al.* Pharmacokinetics and acceptability of once- versus twice-daily lamivudine and abacavir in HIV type-1-infected Ugandan children in the ARROW Trial. *Antiviral therapy* **15**, 1115-24 (2010).

11. Achenbach, C.J., Scarsi, K.K. & Murphy, R.L. Abacavir/lamivudine fixed-dose combination antiretroviral therapy for the treatment of HIV. *Advances in therapy* **27**, 1-16 (2010).
12. Teutonico, D. *et al.* Development of a template for clinical trial simulations in COPD. *Poster presented at PAGE Conference 2010*
13. Tannenbaum, S.J., Holford, N.H.G., Lee, H., Peck, C.C. & Mould, D.R. Simulation of correlated continuous and categorical variables using a single multivariate distribution. *Journal of pharmacokinetics and pharmacodynamics* **33**, 773-94 (2006).
14. Antiretroviral therapy for HIV infection in infants and children. Available at <http://www.who.int/hiv/pub/paediatric/paed-prelim-summary.pdf> (last accessed 7 June 2013)
15. Dickinson, L. *et al.* Sequential population pharmacokinetic modeling of lopinavir and ritonavir in healthy volunteers and assessment of different dosing strategies. *Antimicrobial agents and chemotherapy* **55**, 2775-82 (2011).
16. Kappelhoff, B.S. *et al.* Population pharmacokinetics of efavirenz in an unselected cohort of HIV-1-infected individuals. *Clinical pharmacokinetics* **44**, 849-61 (2005).
17. Zhao, W., Piana, C., Danhof, M., Burger, D., Pasqua, O.D. & Jacqz-Aigrain, E. Population pharmacokinetics of abacavir in infants, toddlers and children. *British journal of clinical pharmacology* (2013).
18. Huang, Y., Rosenkranz, S.L. & Wu, H. Modeling HIV dynamics and antiviral response with consideration of time-varying drug exposures, adherence and phenotypic sensitivity. *Mathematical biosciences* **184**, 165-86 (2003).
19. Bonhoeffer, S., Lipsitch, M. & Levin, B.R. Evaluating treatment protocols to prevent antibiotic resistance. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12106-11 (1997).
20. Wainberg, M.A., Hsu, M., Gu, Z., Borkow, G. & Parniak, M.A. Effectiveness of 3TC in HIV clinical trials may be due in part to the M184V substitution in 3TC-resistant HIV-1 reverse transcriptase. *AIDS (London, England)* **10 Suppl 5**, S3-10 (1996).
21. Wu, H. *et al.* Modeling long-term HIV dynamics and antiretroviral response: effects of drug potency, pharmacokinetics, adherence, and drug resistance. *Journal of acquired immune deficiency syndromes* (1999) **39**, 272-83 (2005).
22. Fletcher, C.V. *et al.* Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. *AIDS (London, England)* **14**, 2137-44 (2000).
23. Melroy, J. & Nair, V. The antiviral activity, mechanism of action, clinical significance and resistance of abacavir in the treatment of pediatric AIDS. *Current pharmaceutical design* **11**, 3847-52 (2005).
24. Best, B.M. *et al.* Efavirenz concentrations in CSF exceed IC50 for wild-type HIV. *The Journal of antimicrobial chemotherapy* **66**, 354-7 (2011).
25. Wein, L.M., D'Amato, R.M. & Perelson, A.S. Mathematical analysis of antiretroviral therapy aimed at HIV-1 eradication or maintenance of low viral loads. *Journal of theoretical biology* **192**, 81-98 (1998).
26. Wu, H. & Ding, A.A. Population HIV-1 dynamics in vivo: applicable models and inferential tools for virological data from AIDS clinical trials. *Biometrics* **55**, 410-8 (1999).
27. Ding, A.A. & Wu, H. Relationships between antiviral treatment effects and biphasic viral decay rates in modeling HIV dynamics. *Mathematical biosciences* **160**, 63-82 (1999).
28. Bangsberg, D.R., Kroetz, D.L. & Deeks, S.G. Adherence-resistance relationships to combination HIV antiretroviral therapy. *Current HIV/AIDS reports* **4**, 65-72 (2007).
29. Tam, L.W.Y. *et al.* The Relationship Between Resistance and Adherence in Drug-Naive Individuals Initiating HAART Is Specific to Individual Drug Classes. *British Columbia Centre for Excellence* **49**, 266-271 (2008).
30. Bachelier, L. *et al.* Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. *Journal of virology* **75**, 4999-5008 (2001).
31. Frost, S.D., Nijhuis, M., Schuurman, R., Boucher, C.A. & Brown, A.J. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. *Journal of virology* **74**, 6262-8 (2000).
32. Ribaud, H.J. *et al.* Pharmacogenetics of plasma efavirenz exposure after treatment discontinuation: an Adult AIDS Clinical Trials Group Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **42**, 401-7 (2006).
33. Bachelier, L.T. *et al.* Human immunodeficiency virus type 1 mutations selected in patients failing efavirenz combination therapy. *Antimicrobial agents and chemotherapy* **44**, 2475-84 (2000).

34. Sosa, N. *et al.* Abacavir and lamivudine fixed-dose combination tablet once daily compared with abacavir and lamivudine twice daily in HIV-infected patients over 48 weeks (ESS30008, SEAL). *Journal of acquired immune deficiency syndromes (1999)* **40**, 422-7 (2005).
35. Parienti, J.-J. *et al.* Not all missed doses are the same: sustained NNRTI treatment interruptions predict HIV rebound at low-to-moderate adherence levels. *PloS one* **3**, e2783 (2008).
36. Cohen, C.J., Colson, A.E., Sheble-Hall, A.G., McLaughlin, K.A. & Morse, G.D. Pilot study of a novel short-cycle antiretroviral treatment interruption strategy: 48-week results of the five-days-on, two-days-off (FOTO) study. *HIV clinical trials* **8**, 19-23 (2007).
37. Dybul, M. *et al.* Long-cycle structured intermittent versus continuous highly active antiretroviral therapy for the treatment of chronic infection with human immunodeficiency virus: effects on drug toxicity and on immunologic and virologic parameters. *The Journal of infectious diseases* **188**, 388-96 (2003).

SECTION V

Conclusions and perspectives

SUMMARY, CONCLUSIONS AND PERSPECTIVES

The development of antiretroviral therapy has been one of the greatest accomplishments of basic and translational research. In the 30 years since the discovery of the HIV virus, 31 medicines have been approved to treat HIV infection and evidence-based guidelines have been developed for their optimal use (1). Initially, in the early 1990s, drugs were administered to HIV-infected patients as monotherapy. Nucleoside monotherapy in previously untreated patients reduced plasma HIV RNA levels by 0.5 to 0.7 log₁₀ copies/mL of plasma. These effects slowly dissipated over months even in the absence of drug resistance, thereby resulting in progressive immunologic decline (2). In the following years it was found that the combination of two antiretroviral nucleosides with relatively modest activities was beneficial in terms of the magnitude and durability of viral suppression. Several nucleoside combinations have resulted in reductions of 1 to 1.5 log₁₀ copies/mL HIV RNA levels in plasma. Even more beneficial were the protease inhibitors (ritonavir, indinavir, and nelfinavir) that produce reductions of HIV RNA levels in the plasma of some patients by as much as 3 log₁₀ copies/mL, with corresponding increases in CD4 levels of 300 to 500 cells/mm³. These findings have contributed to further efforts in establishing the value of therapeutic combinations, with the use of boosted protease inhibitors in the last years, which clearly increased the probability to achieve viral suppression. At the moment, combination antiretroviral therapy with at least three drugs has resulted in substantial reductions in morbidity and mortality in both rich and poor countries. Antiretroviral therapy has been simplified to the point where treatment with a single, multidrug pill once a day is feasible with generally manageable adverse effects (3).

Despite such important improvements, a high number of patients fails to achieve viral suppression. In a marked proportion of HIV-infected individuals on treatment, the virus develops resistance to the currently available drugs. In fact, patients with circulating virus showing resistance to all three classes of conventional antiretroviral drugs is not uncommon. Children are among the group of patients who are most at risk of resistance.

The research performed in this thesis is therefore focused on the treatment of HIV-infected children, a population which presents major challenges compared to adults. Based on the current guidelines for the use of antiretroviral agents in paediatric HIV (4), the most appropriate regimen for an individual child depends on multiple factors, including 1) the age and availability of appropriate drug formulations, 2) the potency, complexity and toxicity of the regimen, 3)

the child and caregiver's ability to adhere to the regimen, 4) the child's personal situation at home and 5) the child's antiretroviral treatment history. Consequently, the use of combination therapy with at least three drugs is recommended for initial treatment of HIV-infected children, including either a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor and a dual nucleoside analogue reverse transcriptase inhibitor backbone. Because antiretroviral therapy will be administered lifelong, the choice of initial antiretroviral regimen should be based on considerations regarding the barriers to adherence, the complexity of schedules and food requirements for different regimens, differences in formulations, palatability problems and potential limitations in subsequent treatment options should resistance develop.

As presented in **Chapter 1**, another important point to consider is the dose rationale for antiretroviral therapy in children. Currently recommended doses have been derived from empirical evidence of clinical response rather than on the pharmacological (virological) properties of the combinations. In addition, the dose of antiretroviral drugs given to children is often based either on weight or body surface area without taking into account in a strictly quantitative manner the underlying physiological changes due to growth, which are known to affect drug pharmacokinetics. Inappropriate exposure, i.e., lower than optimal (sterilising) levels may promote viral mutations which mostly lead to drug resistance. With respect to the disease, an overview of viral characteristics and of the available antiretroviral drug classes currently combined to avoid development of resistance was provided. Taking into account pharmacokinetic and pharmacodynamic properties, we have shown the advantages and limitations of existing methodologies for dosing recommendation. Finally, the role of the patient was scrutinised: a detailed definition of adherence to therapy has been considered together with the main strategies used to enhance treatment compliance in children. Here we highlight the importance of adherence for the development of resistance, which has been shown to differ for each class of antiretroviral drugs.

In this thesis we have explored opportunities to support evidence generation and extrapolation across populations, with special focus on the selection of the **dose**, the optimisation of **dosing regimens** and the impact of patient behaviour towards the treatment, namely **adherence to therapy**. After having identified some of the challenges which need to be overcome to decrease viral failure in children, in **Chapter 2** we propose the use of a model-based approach for the evaluation of covariate effects and forgiveness of non-adherence, which may allow simplification of current dosing regimens taking into consideration inadequate compliance and its implication for efficacy and drug resistance. In conjunction with clinical trial simulations, we aim to demonstrate that it is possible to evaluate relevant clinical scenarios and predict treatment outcome of simplified dosing regimens, taking into account the differences in the patterns of drug intake and the pharmacokinetic-pharmacodynamic properties of antiretroviral drugs. Such factors may all be involved in the development of resistance and thus may ultimately cause viral failure. Our work clearly shows that, even in the event of adequate dosing regimen and dosing frequency, adherence to antiretroviral therapy is crucial to achieve viral suppression.

Although the approval of medicines is primarily determined by empirical evidence generation, situations exist in paediatric diseases in which evidence cannot be generated and inferences from, e.g., underpowered trials, single arm studies, surrogate endpoints or from other populations have to be made to assess the efficacy and safety of a compound. Inferences may also be required to ensure access to treatment and availability of suitable therapeutic regimens to patients. In these circumstances, it has been demonstrated that the assessment of pharmacokinetic-pharmacodynamic relationships in conjunction with modelling and simulation concepts can support dose rationale as well as dose adjustment in specific subgroups of a population. Here we also emphasise that modelling and simulation tools are very useful when ethical issues preclude the possibility to obtain new data and historical data is used as basis for evidence synthesis (5). In fact, throughout this thesis we have used historical data and modelling and simulation to make inferences about the dose and dosing regimens for a range of antiretroviral drugs.

From a conceptual perspective, our approach expands beyond the current paradigm for the evaluation of dosage forms based primarily on pharmacokinetics; it shows how pharmacokinetic-pharmacodynamic relationships can be used as a proxy for efficacy and safety. Our approach also relies on the principles of bridging and extrapolation. As defined in the draft EMA concept paper (6), extrapolation may be generally defined as "extending information and conclusions available from studies in one or more subgroups of the patient population (source population), or in related conditions or with related medicinal products, to make inferences for another subgroup of the population (target population), or condition or product, thus reducing the need to generate additional information (types of studies, design modifications, number of patients required) to reach conclusions for the target population, or condition or medicinal product". On the other hand, from a regulatory perspective the term bridging has been introduced to refer to the generation of data across populations when extrapolation is unsuccessful or not appropriate. Bridging studies are defined by guidelines as supplemental studies performed to provide pharmacokinetic, pharmacodynamic or clinical data on efficacy, safety, dosage, and dose regimen in a new population, region or condition. Obviously, an implicit assumption for such studies is that of pharmacokinetic non-inferiority and therapeutic equivalence when comparable pharmacodynamic effects are observed.

The scientific and clinical questions raised in the various chapters of this thesis illustrate the implementation of the concepts mentioned above. Among other things we have shown that pharmacokinetic-pharmacodynamic relationships can be used as basis for the evaluation of therapeutic equivalence. However, as illustrated for lamivudine and abacavir, a basic requirement must be satisfied, i.e., one needs to ensure the predictive performance of pharmacokinetic models. Of particular interest for paediatric drug research is the role of demographic covariates on drug disposition. In **section 2** we have demonstrated that when defining dosing recommendations in children one should pay attention to the relationships between pharmacokinetic parameters and demographic covariates. The identification of the correct parameter-covariate

relationship has been shown to be particularly challenging in children, especially when the available datasets have limited sample size (i.e., number of patients) or present unbalanced covariate distribution. The role of covariates was then investigated when the model is used for simulation purposes. In particular, we have demonstrated that extrapolations beyond the covariate range used during model building are not feasible in children. Practical recommendations for the use of models for simulation purposes have been provided. In brief, it is concluded that simulations should only be performed after the predictive power of a model has been evaluated.

Based on the evidence of predictive performance, in **section 3** we have presented how modelling and simulation tools can be applied for the evaluation of alternative dosing regimens in a virtual population of HIV-infected children. Such an application is particularly relevant in paediatric HIV, given that simplified dosing regimens with reduced dosing frequency have been shown to improve adherence to therapy. In this section we show that abacavir and lamivudine, two nucleoside-reverse transcriptase inhibitors, can be administered as a once or twice daily dosing regimen without the risk of under or over-exposure in children from 3 months to 12 years old. The possibility to use modelling and simulation to predict the pharmacokinetic properties of a drug in children was then extended to the evaluation of the impact of variable patterns of drug intake and adherence to therapy. In **section 4**, we have explored the implications of partial adherence to antiretroviral treatment outcome. In contrast to the previous section, where pharmacokinetic equivalence underpinned the use of models for bridging and extrapolation purposes, here we have highlighted the importance of pharmacokinetic-pharmacodynamic relationships as the basis for the assessment of efficacy and safety. Extrapolations were performed under the assumption that pharmacokinetic-pharmacodynamic relationships serve as proxy for therapeutic equivalence. This assumption has allowed us to evaluate forgiveness of non-adherence, an important property which reflects the ability of a drug or a regimen to achieve viral suppression despite sub-optimal adherence (7). This concept is illustrated in figures 1-4, which show indistinguishable differences in the pharmacokinetics of lamivudine and abacavir after three-day and two-week interruptions in therapy, irrespective of the dosing regimen.

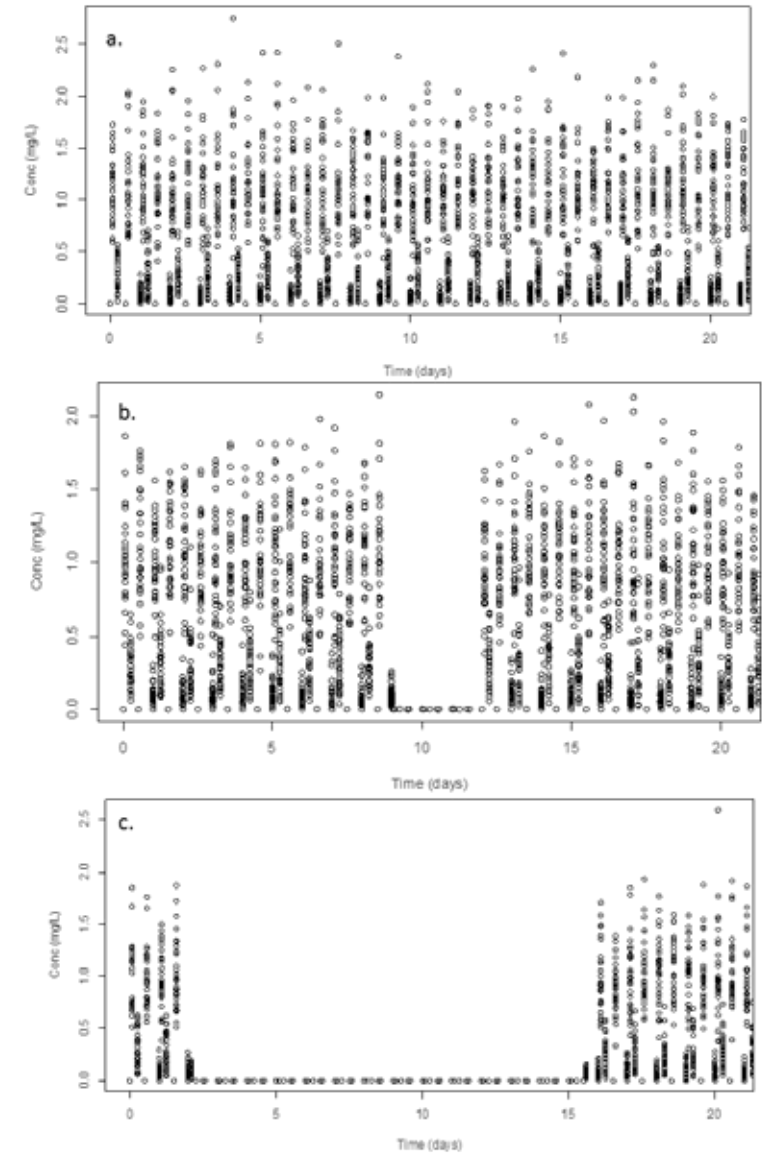


Figure 1 Lamivudine pharmacokinetic profile for twice daily dosing regimen in a hypothetical group of children (n=30) aged between 3 and 12 years. Perfect adherence (a), treatment interruption of three days (b) and two weeks (c) have been simulated.

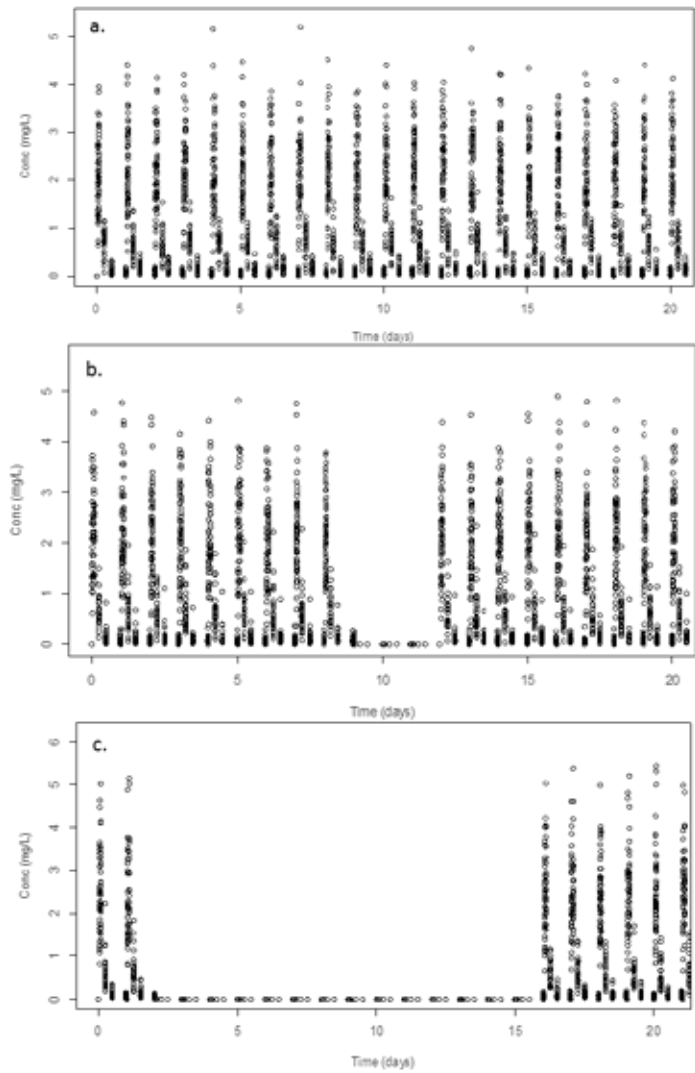


Figure 2 Lamivudine pharmacokinetic profile for once daily dosing regimen in a hypothetical group of children (n=30) aged between 3 and 12 years. Perfect adherence (a), treatment interruption of three days (b) and two weeks (c) have been simulated.

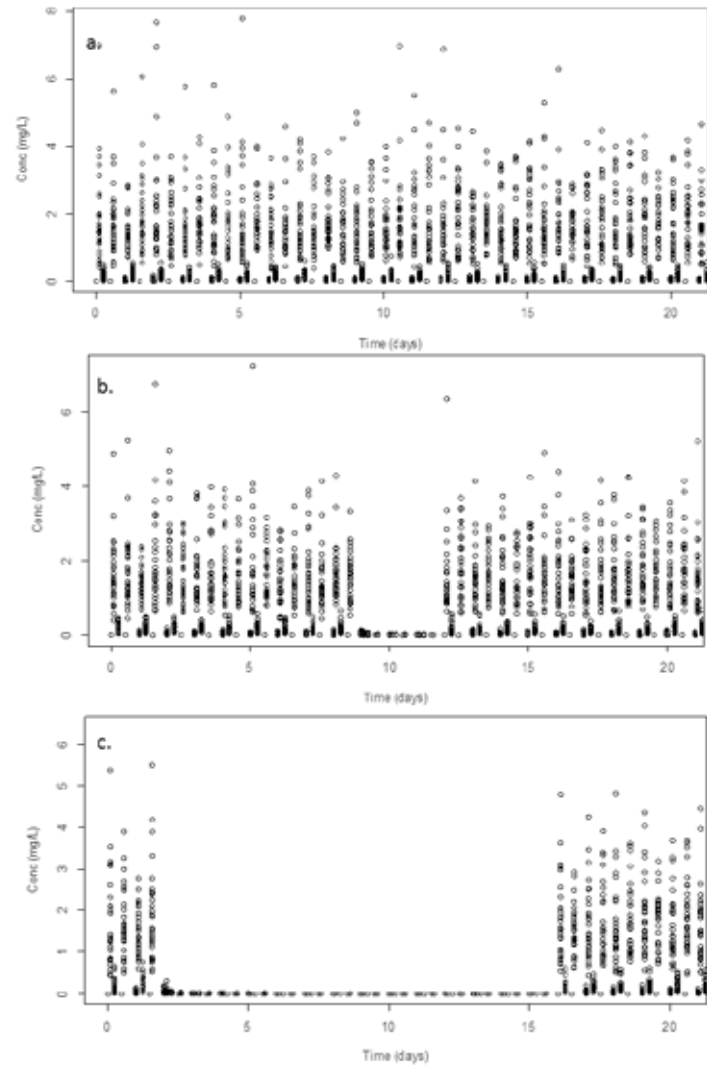


Figure 3 Abacavir pharmacokinetic profile for twice daily dosing regimen in a hypothetical group of children (n=30) aged between 3 and 12 years. Perfect adherence (a), treatment interruption for three days (b) and two weeks (c) have been simulated.

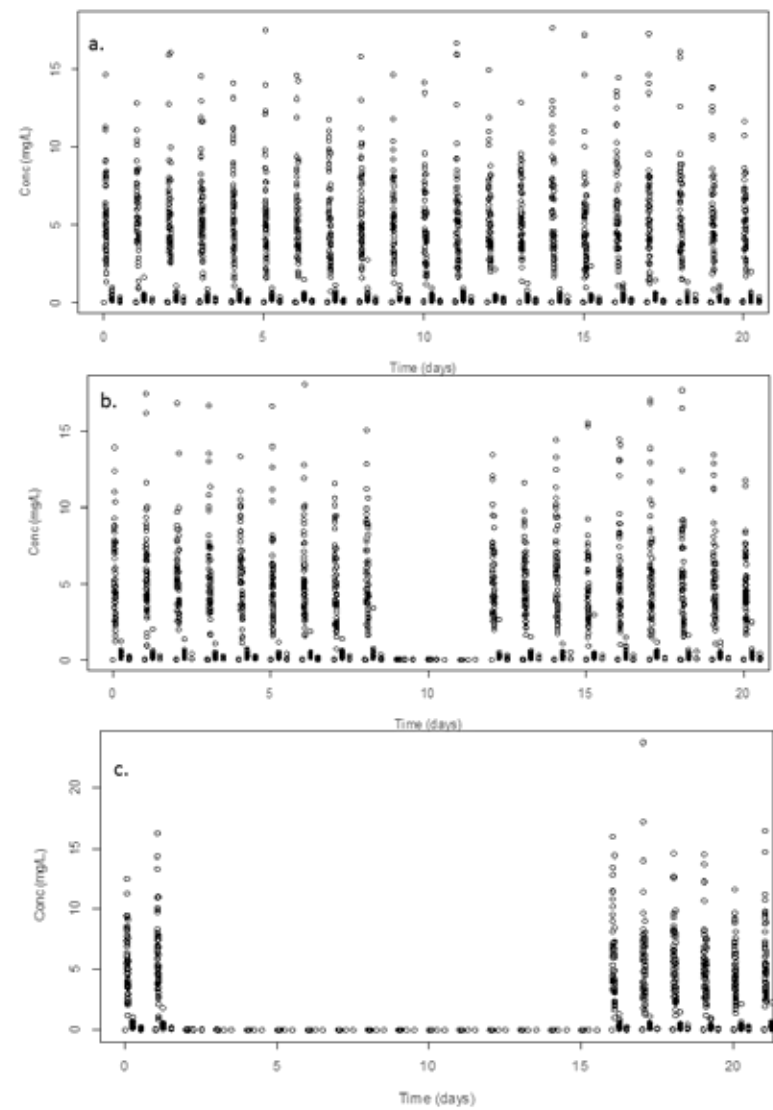


Figure 4 Abacavir pharmacokinetic profile for once daily dosing regimen in a hypothetical group of children (n=30) aged between 3 and 12 years. Perfect adherence (a), treatment interruption for three days (b) and two weeks (c) have been simulated.

Our investigations were performed using a clinical trial simulation template, which allowed for detailed evaluation of relevant scenarios of non-adherence in children, providing important insight into the patterns of non-adherence which should be avoided by the patients. In fact, the opportunity to derive data which would not be attainable in real-life due to obvious ethical and practical reasons constitutes one of the main achievements of this thesis. Furthermore, we have shown that clinical trial simulations also allow assessment of the robustness of alternative dosing regimens in the event of partial adherence. We have found that when both drugs are administered once daily in combination with efavirenz, not only the exposures are comparable but also the forgiveness of non-adherence of the drug combination. This is illustrated in Figure 5, which depicts a clinical trial simulation scenario in which the treatment is interrupted for three consecutive days and for two consecutive weeks.

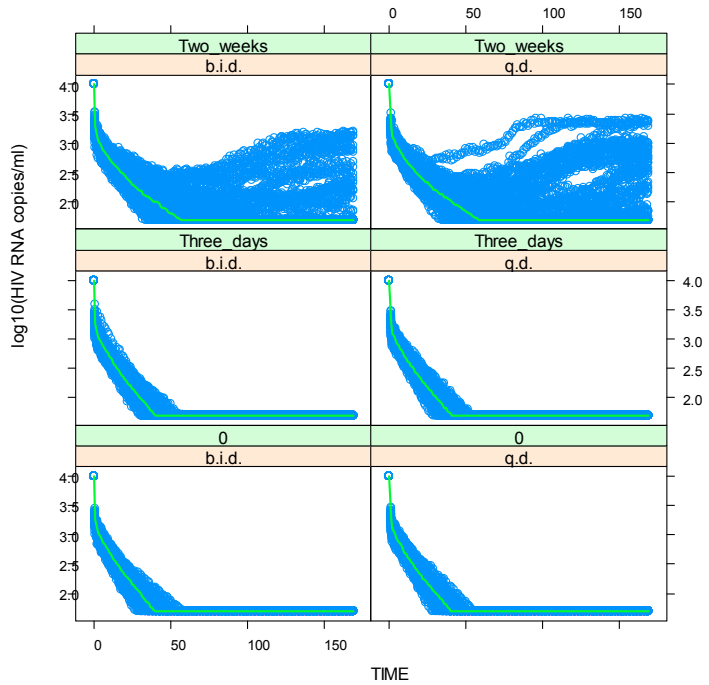


Figure 5 Change in viral load after administration of lamivudine and abacavir in combination with efavirenz as a twice (b.i.d.) (left panel) and once (q.d.) daily dosing regimen (right panel) in a hypothetical group of children (n=30) between 3 and 12 years of age. For comparative purposes, perfect adherence, a three-day treatment interruption and a two-week treatment interruption have been simulated for each regimen.

From a clinical perspective these findings dismiss the concerns regarding the use of a simplified dosing regimen in children, given that the forgiveness of non-adherence is comparable to the currently recommended one.

11.1. IMPACT OF DATASET SIZE AND COVARIATE DISTRIBUTION ON THE PREDICTIVE PERFORMANCE OF PHARMACOKINETIC MODELS IN CHILDREN

One of the primary issues in paediatric clinical pharmacology research is the management of exposure differences attributed to variation in size, age and other developmental factors (8). A model-based approach may be used to systematically evaluate and quantify these differences. However, one has to keep in mind that models misspecifications may lead to serious consequences when conclusions based on these models are used for clinical decision making, such as erroneous dose recommendations. In **chapter 3** we performed a thorough assessment of the pharmacokinetics of lamivudine in children, with focus on the correlation between pharmacokinetic parameters and demographic covariates. A meta-analysis was performed, in which data from three clinical trials in children between 3 months and 12 years old were combined. We found that lamivudine was best described by a one compartment model with body weight exponentially correlated to clearance and volume of distribution. Interestingly, we show that such relationships can be identified only when the three datasets were analysed together. This finding emphasises a very important concept in paediatric pharmacokinetic modelling: sample size is critical when assessing parameter-covariate relationships in children. These results clearly suggest that, although a model built using a small population may be suitable for descriptive purposes its use for predictive purposes in a new population should be avoided. In addition, our findings reveal the implications of unbalanced covariate distributions. Unbalanced covariate distributions may introduce model misspecification which may easily compromise model-based predictions in a new population. Such findings were confirmed in **chapter 4**, in which a population pharmacokinetic analysis was performed in children receiving abacavir, an antiretroviral drug often given in combination with lamivudine. Again, in this chapter we showed the importance of a meta-analytical approach to investigate the pharmacokinetics of a drug in children. Furthermore, our work highlights the clinical application of model-based data analysis. Using simulation tools, we showed that drug exposure can be predicted even in case of very sparse sampling, which has always been a concern for drug therapeutic monitoring.

The importance of the correlation between pharmacokinetic parameters and covariates in children and its impact on the predictive performance of a model has been further evaluated in **chapter 5**. In this chapter we use simulations to explore whether drug exposure can be predicted for a population belonging to a different age range than the ones used for model building. Clearly, this attempt failed, indicating that pharmacokinetic models should not be used to perform extrapolations beyond the covariate range available during model building. Predictions from pharmacokinetic models are reliable only in case of interpolations involving a subgroup of the initial population. In addition, we showed that the covariate point estimates (e.g. median) used to describe the mathematical relationship between covariate and pharmacokinetic param-

eters should be fixed even when interpolations are referred to a subset of the initial population.

In summary, this section shows the importance of demographic factors on the pharmacokinetics of two drugs currently administered as first-line therapy in HIV-infected children. In addition, our findings highlight some critical issues for covariate selection. We recommend therefore the use of somewhat larger sample sizes than what is currently done in clinical practice. Ultimately we have shown the importance of balanced covariate distribution and its implications for the predictive performance of a pharmacokinetic model: a model in children should not be used for extrapolation beyond the covariate range explored during model building. This finding raises further questions about the widespread use of allometric scaling concepts in paediatric research.

11.2. EVALUATION OF SIMPLIFIED ANTIRETROVIRAL DOSING REGIMENS IN CHILDREN

The possibility to administer an antiretroviral drug according to a once daily dosing regimen in children is certainly very appealing. However, one needs to be entirely confident that drug exposure achieved after once daily dosing is comparable to historical values achieved after twice daily dosing. In the third section of this thesis we show the use of a model-based approach to evaluate the feasibility of an alternative dosing regimen for lamivudine, which is currently administered as once daily in adults and twice daily in children. In **chapter 6** we apply simulations to assess whether lamivudine could be administered as once daily dosing in a virtual group of children between 3 months and 12 years old. The pharmacokinetic model developed and validated previously in chapter 3 was used for this purpose. Evidently, once daily doses of lamivudine leads to AUC_{0-24} and peak concentrations comparable to historical values in children who received a twice daily dosing regimen. The same simulation exercise was performed for abacavir, which is also administered as once daily in adults and twice daily in children. In **chapter 7** we used a population pharmacokinetic model developed in chapter 4 to evaluate whether the exposure of abacavir following a once daily dosing regimen was comparable to the current regimen. Again it was demonstrated that once daily doses of abacavir in children leads to AUC_{0-24} and peak concentration values comparable to those observed in historical data in children and adults.

Such results are crucial for the improvement of antiretroviral therapy in children, given that once daily doses may improve acceptability and adherence to therapy. In addition, our approach illustrates how to explore hypothetical scenarios in virtual patients. This concept is particularly useful in paediatric clinical pharmacology research. One of the main advantages is the possibility to evaluate treatment response in a large number of children, without exposing them to the actual treatment. Further details on the implementation of virtual clinical trials are discussed and expanded in the next section of this thesis.

11.3. ADHERENCE TO HIV THERAPY: “DRUGS DO NOT WORK IN PATIENTS WHO DO NOT TAKE THEM”

It is well known that the continuity of drug action derives from the continuity of execution of a prescribed dosing regimen, which is aimed at yielding pharmacologically suitable exposure to the active moiety between successive doses. Treatment interruptions can alter drug action, but the consequences of interruptions in dosing may vary according to the drug, the length of interruption and the disease being treated (9). In this context, the sentence attributed to former US Surgeon General C. Everett Koop, “Drugs don’t work in patients who don’t take them”, summarises perfectly the importance of patient adherence to therapy.

However, to fully understand the implications of the factors determining drug response and variability, one needs a comprehensive approach that takes into account the effects of developmental growth as well as other patient-specific characteristics which could modify drug pharmacokinetics. In addition, it is important to recognise how these factors interrelate with the proposed dosing regimen or interval. Current mainstream views on the use of fixed-dose combinations suggest that reduced dosing frequency improves antiretroviral therapy. Yet, evidence of therapeutic equivalence cannot be derived from bioequivalence studies or simply by comparison of pharmacokinetic data alone. In HIV therapy, the benefit of drug combinations results primarily from pharmacodynamic interactions. Accurate evaluation of changes in dosing regimen requires therefore an assessment of the impact of patient adherence on pharmacodynamics. Differences in the patterns of drug intake are a major cause of unintended variability in drug exposure, which have been associated with the failure of treatment (10). Here we have shown how to quantitatively evaluate the role of partial adherence to therapy. In other words, we have provided the framework for addressing an important clinical question: “How many doses can a patient miss without affecting treatment outcome?”

Modelling as well as other statistical approaches have been previously used to investigate the impact of adherence on therapeutics, which are worth mentioning. In fact, the consequence of partial adherence to antiretroviral therapy is one of the focus areas in this research field. For instance, Pfister *et al.* integrated adherence information as model covariate to determine its effect on drug exposure (11). Subsequently longitudinal models have been proposed to estimate the effect of patient adherence on the rate at which the patients progress through the HIV infection. Other investigators have considered the use of Markov models and other stochastic methods (12–17). Among other advantages, the approach allows the response at any time point to be conditional on that observed in the previous time period. The novelty of our work lies in the fact that for the first time the effect of imperfect adherence has been evaluated using a framework in which all the elements of a clinical trial simulation were combined. Compared to the abovementioned studies, important elements were introduced which were missing previously, including the use of combination therapy, more realistic patterns of non-adherence (ran-

dom drug holidays, imperfect timing of successive doses) and a correlation between adherence and drug resistance. To illustrate the usefulness of such an integrated approach, three scenarios were selected for evaluation in this thesis: a single drug used as monotherapy, a cocktail consisting of three drugs and a fixed dose combination of three drugs administered according to a simplified dosing regimen. Our analysis also included a relationship between adherence and the probability to develop drug resistance. This clinical trial simulation framework represents an important improvement in that it encompasses a combination of various mathematical models, allowing for the prediction of treatment outcome for scenarios that cannot be assessed empirically in a controlled, randomised manner.

In **chapter 8**, the forgiveness of non-adherence of the three different classes of antiretroviral drugs currently used as first-line therapy in children (NNRTI, NRTI and boosted PI) has been evaluated. This study represents the first attempt to predict treatment outcome using a combined approach in which pharmacokinetic, pharmacodynamic and disease models are applied concurrently. In addition, the use of a logistic regression to describe the relationship between the number of days in which the drug exposure remains below the expected therapeutic levels and the probability to develop drug resistance provided the basis for linking partial adherence and viral failure. For practical purposes and ease of interpretation, we have parameterised adherence in terms of the patterns of doses not taken rather than as percentage of doses taken. It is essential to mention that for predictive purposes, all the models used here have been previously evaluated and validated. Our findings provide a preliminary indication about the level of adherence required by each antiretroviral drug to prevent viral failure when used as monotherapy. In reality, these scenarios represented a starting point to characterise the implications of non-adherence or partial adherence when a combination of two or more drugs is used. Subsequently, in **chapter 9**, a clinical trial simulation was performed in which a cocktail of three drugs is administered to a population of HIV-infected children. Our results reveal that for a NNRTI-based regimen long periods of treatment interruption may represent a higher risk of viral failure than when the same number of doses is missed randomly.

The aforementioned findings indicate that the pharmacokinetic-pharmacodynamic relationships of antiretroviral drugs may be used to predict treatment efficacy in the investigation of forgiveness of non-adherence and, consequently, of simplified dosing regimens. In fact, pharmacokinetic-pharmacodynamic relationships may be considered a “proxy” of therapeutic equivalence in the assessment of forgiveness of non-adherence, in an analogous manner as pharmacokinetics in bioequivalence studies. Conceptually, this offers the possibility to replace efficacy trials by pharmacokinetic-pharmacodynamic studies. As shown in **chapter 10**, the effect of partial adherence to a simplified dosing regimen was evaluated using the underlying pharmacokinetic-pharmacodynamic relationships of lamivudine and abacavir in combination with efavirenz. Our results reveal that a once daily regimen yields comparable forgiveness of non-adherence as the currently approved twice daily in all the scenarios of non-adherence. In

practical terms, this means that a reduction in dosing frequency can ultimately reduce pill-burden in children without increasing the risk of resistance.

In summary, the clinical trial simulation framework used in chapters 8, 9 and 10, provide the opportunity to evaluate clinically relevant information about the level of adherence required to prevent viral failure and, if extended to other treatment options, may enable physicians to consider the best drug regimen based on historical adherence patterns of the patient. It is also worth mentioning that dropout information was not included in the simulations to ensure appropriate characterisation of the impact of poor adherence without the confounding effects of dropout. However, our findings suggest that poor adherence may have equal, if not more influential, effects on treatment outcome as dropout.

11.4. CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

The results presented in this thesis highlight the value of a model-based approach to address important issues in paediatric HIV research, which have remained unaddressed despite the ongoing scientific and clinical progress in the field. The first point regards the dose recommendation for children based on bridging concepts, rather than on body size. This implies the characterisation of developmental factors influencing drug exposure in the target population, so that exposure attained in children is comparable to adults. In addition, our work has shown the potential limitations of modelling and simulation methodologies for the prediction of pharmacokinetics in children. We have therefore delineated the following recommendations for the implementation of a model-based approach in paediatric research:

- 1) Population pharmacokinetic studies must consider suitable inclusion or stratification criteria to ensure balanced covariate distribution;
- 2) The use of meta-analysis including different population subgroups is strongly encouraged to prevent model misspecification due to the confounding effects of unbalanced covariates;
- 3) The predictive performance of a model is limited to interpolations. The accuracy of extrapolations beyond the range of the population used for model building cannot be warranted even when diagnostic and model validation procedures show evidence of goodness-of-fit.
- 4) Extrapolation across populations beyond the conditions or population under investigation requires the use of models for simulation, which are not the same as models for estimation, as in the case of models developed using the maximum likelihood.
- 5) Models for simulation are essential for hypothesis generation and inferences. They provide insight into conditions and scenarios which may not be observed or controlled in real life. As such, they can be parameterised in term of drug, patient and system-specific parameters, irrespective of precision or identifiability, which are data driven.

Based on the aforementioned recommendations, the use of a model-based approach can be extended beyond the issues regarding the dose rationale. It can be applied systematically for the evaluation of simplified treatment options, which is very appealing in paediatric research. Undoubtedly, in conjunction with clinical trial simulations, models become an experimental design tool.

Despite our efforts to incorporate non-adherence into the analysis, several issues were encountered, which need to be highlighted as they impose additional assumptions for model parameterisation. One of these assumptions refers to the relationship between adherence and the probability to develop resistance. We have noticed that the data on adherence available in literature are expressed as percentage of doses taken, which did not meet our objectives. Instead, we have used a logistic regression to describe that relationship. This adaptation brings to light the need for more precise and continuous data on patient adherence. In fact, this situation calls for the systematic use of medication event monitoring systems (MEMs), currently the most reliable measure of patient adherence. Moreover genotyping should be carried out more frequently to gain insight in the correlation between adherence and probability to develop drug resistance.

We are also aware about the impossibility to validate the results from our clinical trial simulation with a prospective trial. In this respect, we can conclude that forgiveness of non-adherence is better defined by means of a not-in-trial simulation (NITS), a concept which has been recently applied for the assessment of pro-arrhythmic risk (18). In contrast to typical clinical trial simulations, this methodology makes use of simulations to characterise the role of design factors which have been omitted from a randomised trial; in our case we have characterised patient adherence to therapy, which cannot be explored in a controlled manner for ethical reasons.

Not-in-trial-simulations constitute therefore a promising starting point for the evaluation of design factors or patient characteristics which cannot be reproduced in real life. Most importantly, this feature opens new opportunities for the evaluation of effectiveness of the treatment. In HIV research, we envisage the prospect for exploring the thresholds of non-adherence which should be avoided to prevent viral failure. In addition, other therapeutic areas, such as e.g., tuberculosis, oral contraceptives or hormonal replacement, would greatly benefit from the concepts presented here (19). In tuberculosis, an *in vitro* model was used to evaluate, for the first time, the forgiveness of a standard tuberculosis drug regimen with the objective to determine whether poor adherence, in the form of simulated drug holidays, would lead to incomplete sterilisation and/or emergence of drug resistance.

Interestingly, several attempts have been made to quantify forgiveness of non-adherence to oral contraceptive drugs. Despite the use of empirical protocols, quantitative evaluation of non-adherence in this area has been based on study designs that include controlled, blinded substitution of placebo for active drug, with frequent measurements of drug action to determine how long it takes, after the last-taken dose of active drug, for the drug's actions to wash-out. Such a study design, known as the placebo substitution-for-active (PSA), has guided the first efforts to write evidence-based instructions for patients on what to do if they miss one or

more pills (9). PSA designs have also been applied in the evaluation of non-adherence in depression by Rosenbaum *et al.*, who carried out a 5- to 8-day placebo substitution study in patients treated with fluoxetine, sertraline, or paroxetine (20).

Obviously, such a trial design could not be applied in antiretroviral therapy and other serious, fatal diseases. The use of NITS methodology, as developed in this thesis, represents a novel and alternative approach to evidence synthesis. Eventually, this will allow information on forgiveness of non-adherence to be systematically included in the summary of product characteristics, providing guidance for prescribers and patients on how to minimise the consequences of missed doses and define the course of action to correct any deviation from the prescribed dosing regimen. Another important aspect that pertains to risk management is the possibility to evaluate prospectively the forgiveness of non-adherence. In other words, NITS can be used to understand what happens in real life irrespective of the evidence derived during a clinical trial, which often shows higher degree of adherence as compared to day-to-day treatment.

The assumption that evidence generation is a sine qua non condition for regulatory approval and optimisation of the therapeutic use of medicines needs to be revisited. The hurdles for protocol implementation and feasibility issues in paediatric research and other serious infectious diseases raise questions about the relevance of evidence generated under strict controlled conditions, as they do not reproduce or reflect real life use of medicines. It should be noted that the reliance on empirical evidence is kindled by regulatory agencies, which continue to issue guidelines that outline the requirements for the approval of medicines based on evidence generation. By contrast, formal acknowledgement of the role of evidence synthesis as the basis for the evaluation of efficacy and safety has not been fully embraced in clinical therapeutic practice or by the regulatory approval process, despite its widespread application in the evaluation of effectiveness and cost-benefit analyses.

A shift in paradigm is required in which evidence synthesis is favoured, making evidence generation a confirmatory step in the continuum between assumptions and empirical evidence. Among the lessons learned from the work presented throughout this thesis, it is worth emphasising that evidence generation without data integration, including systematic incorporation of prior knowledge leads to less than optimal experimental protocols and potentially inappropriate decision criteria. This aspect is often ignored in the rationale for mainstream paediatric clinical trials. Another important point arising from our examples is that evidence synthesis can be far more powerful than evidence generation, as it gives insight into conditions that cannot be evaluated experimentally.

We finish this thesis by evoking the very initial question in its title, i.e., does half a day make a difference to treatment outcome? Based on evidence synthesis by modelling and simulation it can be concluded that it does not. Moreover, it is our expectation that this work will contribute to consolidating the use of pharmacokinetic-pharmacodynamic relationships as the basis for inferences about therapeutic equivalence, thereby overcoming the need to perform unnecessary efficacy trials in children.

References

1. A. Fauci "After 30 years of HIV/AIDS, real progress and much left to do," Washington Post (2011).
2. Havlir, D.V. & Richman, D.D. Viral dynamics of HIV: implications for drug development and therapeutic strategies. *Annals of internal medicine* **124**, 984-94 (1996).
3. Folkers, G.K. & Fauci, A.S. Controlling and ultimately ending the HIV/AIDS pandemic: a feasible goal. *JAMA : the journal of the American Medical Association* **304**, 350-1 (2010).
4. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. Available at <http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf>.
5. Harnisch, L. &, Shepard, G.P. and Della Pasqua, O. Modeling and Simulation as a Tool to Bridge Efficacy and Safety Data in Special Populations. CPT: Pharmacometrics & Systems Pharmacology (2013)
6. European Medicines Agency. Concept paper on extrapolation of efficacy and safety in medicine development. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500 (2013).
7. Shuter, J. Forgiveness of non-adherence to HIV-1 antiretroviral therapy. *The Journal of antimicrobial chemotherapy* **61**, 769-73 (2008).
8. Barrett, J.S., Labbé, L. & Pfister, M. Application and impact of population pharmacokinetics in the assessment of antiretroviral pharmacotherapy. *Clinical pharmacokinetics* **44**, 591-625 (2005).
9. Osterberg, L.G., Urquhart, J. & Blaschke, T.F. Understanding forgiveness: minding and mining the gaps between pharmacokinetics and therapeutics. *Clinical pharmacology and therapeutics* **88**, 457-9 (2010).
10. Kenna, L. a, Labbé, L., Barrett, J.S. & Pfister, M. Modeling and simulation of adherence: approaches and applications in therapeutics. *The AAPS journal* **7**, E390-407 (2005).
11. Pfister, M. *et al.* Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group Study 398. *Antimicrobial agents and chemotherapy* **47**, 130-7 (2003).

12. Vrijens, B., Goetghebeur, E., de Klerk, E., Rode, R., Mayer, S. & Urquhart, J. Modelling the association between adherence and viral load in HIV-infected patients. *Statistics in medicine* **24**, 2719-31 (2005).
13. Labbé, L. & Verotta, D. A non-linear mixed effect dynamic model incorporating prior exposure and adherence to treatment to describe long-term therapy outcome in HIV-patients. *Journal of pharmacokinetics and pharmacodynamics* **33**, 519-42 (2006).
14. Girard, P., Blaschke, T.F., Kastrissios, H. & Sheiner, L.B. A Markov mixed effect regression model for drug compliance. *Statistics in medicine* **17**, 2313-33 (1998).
15. Lou, J. & Smith, R.J. Modelling the effects of adherence to the HIV fusion inhibitor enfuvirtide. *Journal of theoretical biology* **268**, 1-13 (2011).
16. Miron, R.E. & Smith, R.J. Modelling imperfect adherence to HIV induction therapy. *BMC infectious diseases* **10**, 6 (2010).
17. Podsadecki, T.J., Vrijens, B.C., Tousset, E.P., Rode, R.A. & Hanna, G.J. "White coat compliance" limits the reliability of therapeutic drug monitoring in HIV-1-infected patients. *HIV clinical trials* **9**, 238-46 (2008).
18. Chain, A., Dieleman, J., Danhof, M., Sturkenboom, M., Della Pasqua, O. Not-in-trial simulation II: Predicting cardiovascular risk from clinical trial data. *British Journal of Clinical Pharmacology* - In press (2013).
19. Dartois, V. Drug forgiveness and interpatient pharmacokinetic variability in tuberculosis. *The Journal of infectious diseases* **204**, 1827-9 (2011).
20. Rosenbaum, J.F., Fava, M., Hoog, S.L., Ascroft, R.C. & Krebs, W.B. Selective serotonin reuptake inhibitor discontinuation syndrome: a randomized clinical trial. *Biological psychiatry* **44**, 77-87 (1998).

NEDERLANDSE SAMENVATTING (SYNOPSIS IN DUTCH)

Het chronische karakter van de behandeling impliceert toediening van verschillende geneesmiddelen gedurende de volledige kinderleeftijd. Daarnaast zijn aanpassingen van de dosering vaak nodig om te verzekeren dat de juiste blootstelling wordt bereikt en behouden gedurende de behandelperiode. Fysiologische groei en ontwikkeling in pediatrische patiënten kunnen significante invloed hebben op ADME processen, in het bijzonder de opname en de uitscheiding van het geneesmiddel. Onrijpe nierfunctie, afwijkende hepatische enzymactiviteit en verschillen in opname van het medicijn leiden tot variaties in de systemische blootstelling van antiretrovirale geneesmiddelen bij kinderen. Het chronische karakter van de behandeling impliceert toediening van verschillende geneesmiddelen gedurende de volledige kinderleeftijd. Daarbij zijn aanpassingen van de dosering vaak nodig om te verzekeren dat de juiste blootstelling wordt bereikt en behouden gedurende de gehele behandelperiode. Fysiologische groei en orgaanontwikkeling in pediatrische patiënten kunnen significante invloed hebben op de absorptie, de distributie, de metabole afbraak en de excretie van een geneesmiddel (de zgn. ADME processen). Dat betreft in het bijzonder de opname en de uitscheiding. Onrijpe nierfunctie, afwijkende hepatische enzymactiviteit en verschillen in opname van het medicijn leiden tot variaties in de systemische blootstelling van antiretrovirale geneesmiddelen bij kinderen.

Vanuit het therapeutische invalshoek ziet men een groeiende hoeveelheid bewijs van de complexiteit van therapietrouw in HIV geïnfecteerde kinderen, waarbij huidige niveaus vaak suboptimaal zijn. Lage therapietrouw kan het resultaat van de behandeling negatief beïnvloeden, zelfs wanneer men met de toegediende dosis rekening houdt en deze aanpast op basis van demografische eigenschappen en leeftijds-gerelateerde veranderingen. Dit kan leiden tot ontwikkeling van resistentie tegen het merendeel van de geneesmiddelen, wat uiteindelijk leidt tot falen van de antivirale behandeling. Therapietrouw wordt beïnvloed door veel factoren, die verdeeld kunnen worden in eigenschappen van het kind, de hulpverlener(s) en het gezin, de formulering en het doseringsschema. Doordat kinderen overwegend afhankelijk zijn van hun ouders of verzorger bij het toedienen van medicatie, kan een frequente doseringsschema een uitdaging zijn. In deze gevallen kan het verlagen van de het aantal doseringen cruciaal zijn bij het verbeteren van de therapietrouw. Echter, men moet bedacht zijn op het feit dat een vereenvoudigd doseringsschema het risico op een te lage of te hoge blootstelling kan verhogen. De beoordeling hiervan is nog gecompliceerder doordat de werkzaamheid en veiligheid in patiënten ingeschat

moet worden voor combinatietherapieën, waarbij de bijdrage in het effect van elk individueel medicijn vaak moeilijk te bepalen is.

Bovengenoemde in ogenschouw nemende is het moeilijk te begrijpen waarom de keus voor dosering en doseringsschema's voor de behandeling van pediatrische HIV patiënten gestuurd blijft worden vanuit empirische beslissingen. Een integrale benadering is vereist, waarin de evaluatie van werkzaamheid en veiligheid niet alleen bepaald wordt door bewijs uit klinische studies in kinderen, maar ook met gevolgtrekkingen vanuit historische data en kwantitatieve farmacologische concepten.

Het doel van het onderzoek in dit proefschrift is de ontwikkeling van nieuwe benaderingen voor de vergelijking van de werking van geneesmiddelen tussen populaties, met speciale aandacht voor de selectie van de juiste dosis en het juiste doseringsschema in met HIV geïnfecteerde kinderen. We stellen het gebruik van een modelmatige benadering voor, die het mogelijk maakt de oorzaken van variatie in de blootstelling van geneesmiddelen in kinderen vast te stellen, rekening houdend met de interactie tussen therapietrouw en de eigenschappen van antiretrovirale geneesmiddelen. Hiernaast laten we het nut van simulaties van klinische studies en de rol van gesimuleerde scenario's zien, waardoor bekende problemen bij pediatrisch onderzoek voorkomen kunnen worden. Hierin worden de gevolgen van veranderingen in farmacokinetiek en farmacodynamiek beoordeeld die samenhangen met verschillen in dosering, doseringsschema en verschillende patronen van therapietrouw. Deze factoren kunnen niet op een ethisch aanvaardbare wijze in de klinische praktijk worden geëvalueerd.

Drie kernvragen zullen de basis vormen voor het werk dat gepresenteerd wordt in de volgende hoofdstukken van dit proefschrift:

1. Hoe kan de juiste dosering geselecteerd worden, rekening houdend met het feit dat veranderingen in blootstelling als gevolg van groei tijdens ontwikkeling vaak niet-lineair gecorreleerd zijn met de lichaamsgrootte en dat bewijs vanuit klinische studies gebaseerd is op waarnemingen in kleine, niet gebalanceerde cohorten?
2. Kan het doseringsschema bij kinderen aangepast worden op basis van farmacokinetischfarmacodynamische en farmacokinetische-farmacodynamische modellen en overbruggingsconcepten?
3. Kunnen farmacokinetische-farmacodynamische relaties gebruikt worden om de invloed van verschillende patronen van therapietrouw op de behandeluitkomst op een kwantitatieve wijze te evalueren, omdat dit niet formeel getest kan worden in de klinische praktijk vanwege ethische bezwaren?

Het is ons streven om aan te tonen hoe het gebruik van een modelmatige benadering de mogelijkheid geeft om de invloed van verschillen in farmacokinetiek op de farmacodynamiek te

beoordelen, om vervolgens de behandeluitkomst (bijvoorbeeld virologisch falen) in kinderen te voorspellen, alvorens ze bloot te stellen aan een klinisch protocol. Uiteindelijk zullen we aantonen dat de aanname van een vergelijkbare relatie tussen blootstelling en respons in volwassenen en kinderen het niet alleen mogelijk maakt om tijdens de selectie van de dosering rekening te houden met verschillen in fysiologische processen veroorzaakt door groei en ontwikkeling die tot veranderingen in farmacokinetiek leiden, maar dat deze aanname ook de basis geeft voor evaluatie van de invloed van veranderingen in het doseringsschema.

12.1. ALGEMENE INLEIDING

Zoals is aangetoond in **hoofdstuk 1** is de motivering achter de dosering van antiretrovirale therapie in kinderen een cruciaal punt ter overweging. De huidige doseringsadviezen zijn eerder afgeleid uit empirisch bewijs van klinische respons dan gebaseerd op de farmacologische of virologische eigenschappen van de geneesmiddelcombinaties. Daarbij is de bij kinderen toegediende dosering van antiretrovirale geneesmiddelen vaak gebaseerd op gewicht of lichaamsoppervlakte zonder dat op een strikt kwantitatieve manier rekening wordt gehouden met de onderliggende fysiologische veranderingen, waarvan bekend is dat deze de farmacokinetiek beïnvloeden. Hierbij is belangrijk dat een te lage blootstelling mutaties in de hand kan werken, mogelijk leidend tot resistentie. Voor ons onderzoek hadden wij een overzicht van huidige combinatietherapieën tezamen met eigenschappen van verschillende virale subklassen tot onze beschikking. De farmacokinetische en farmacodynamische eigenschappen in acht nemend hebben we de voordelen en beperkingen van bestaande methodologieën voor de selectie van de dosering laten zien. Ten slotte is de rol van de patiënt bij het vaststellen van de behandeluitkomst onderzocht: we bekeken in het licht van een gedetailleerde definitie van therapietrouw de meest gebruikte strategieën om therapietrouw bij kinderen te verhogen. Hiermee onderstrepen we het belang van therapietrouw ter bestrijding van resistentieontwikkeling die, zoals is aangetoond, verschillend is voor elke klasse van antiretrovirale geneesmiddelen.

Daarnaast hebben we in dit proefschrift de mogelijkheden onderzocht om bewijsvorming en extrapolaties tussen populaties te versterken, met speciale aandacht voor de selectie van de **dosering**, de optimalisatie van het **doseringsschema** en invloed van patiëntgedrag aangaande de behandeling, namelijk **therapietrouw**. Na de uitdagingen geïdentificeerd te hebben die overwonnen zullen moeten worden om virologisch falen in kinderen te verlagen, wordt in **hoofdstuk 2** het gebruik van een modelmatige benadering voor de evaluatie van effecten van covariaten en de vergevingsgezindheid van therapieontrouw beschreven. Met deze benadering kunnen de huidige doseringsschema's worden vereenvoudigd, rekening houdend met mogelijk onvoldoende therapietrouw en de gevolgen hiervan voor zowel effectiviteit van de behandel-

ing en de ontwikkeling van resistentie. Door middel van simulaties van klinische studies zullen we laten zien dat de uitkomst van aangepaste doseringsschema's in relevante scenario's kan worden voorspeld, waarbij de invloed van de therapietrouw en de farmacokinetische-farmacodynamische eigenschappen in ogenschouw worden genomen. Deze factoren zouden betrokken kunnen zijn bij de ontwikkeling van resistentie en kunnen de oorzaak zijn van virologisch falen. Dit werk laat helder zien dat, zelfs in het geval van een adequaat doseringsschema en doseringsfrequentie, de compliantie cruciaal is om onderdrukking van HIV te bereiken.

12.2. INVLOED VAN DE GROOTTE VAN DE POPULATIE (STEEKPROEF) EN DE VERDELING VAN COVARIATEN OP HET VOORSPELLEND VERMOGEN VAN FARMACOKINETISCHE MODELLEN IN KINDEREN

Een van de primaire problemen in klinisch farmacologisch onderzoek in kinderen is hoe om te gaan met verschillen in blootstelling die het gevolg zijn van variaties in grootte, leeftijd en andere factoren die tijdens ontwikkeling een rol spelen. Een modelmatige benadering kan gebruikt worden om deze verschillen systematisch te evalueren en kwantificeren. Echter, men moet zich realiseren dat misspecificaties in het model kunnen leiden tot ernstige gevolgen wanneer de op deze modellen gebaseerde conclusies gebruikt worden voor klinische besluitvormingen als doseringsadviezen. In **hoofdstuk 3** hebben we de farmacokinetiek van lamivudine in kinderen grondig beoordeeld, met een focus op het vaststellen van de correlatie tussen farmacokinetische parameters en demografische covariaten. Wij hebben een meta-analyse verricht waarbij data van drie klinische studies werden gecombineerd. De leeftijd van de in deze studie geïncloseerde kinderen varieerde van 3 maanden tot 12 jaar. De farmacokinetiek van lamivudine werd het best beschreven door een één-compartimentsmodel met lichaamsgewicht exponentieel gecorreleerd aan klaring en het verdelingsvolume. Hierbij viel op dat deze relaties alleen geïdentificeerd konden worden wanneer de drie studies gezamenlijk geanalyseerd werden. Dit benadrukt een concept dat erg belangrijk is bij het modelleren van farmacokinetiek in kinderen: de grootte van de studiegroep is beslissend wanneer de relatie tussen parameter en covariaat bij kinderen beoordeeld moet worden. Verder laat dit onderzoek duidelijk zien dat, hoewel een op een kleine populatie gebaseerd model een goede beschrijving kan geven van deze specifieke groep, men terughoudend moet zijn met voorspellingen in een nieuwe populatie. Hiernaast tonen onze resultaten aan hoe belangrijk de verdeling van covariaten kan zijn. Een ongebalanceerde verdeling kan een misspecificatie in het model introduceren, die leidt tot een beperkte voorspellende waarde in nieuwe populaties. Dit werd bevestigd in **hoofdstuk 4**, waarin een populatie farmacokinetische analyse werd verricht bij kinderen die abacavir toegediend kregen; een antiretroviraal geneesmiddel dat vaak in combinatie met lamivudine gegeven wordt. Ook hier wordt het belang

van een meta-analytische benadering onderstreept bij onderzoek naar de farmacokinetiek van een geneesmiddel in kinderen. Verder illustreert ons werk de klinische toepasbaarheid van een modelmatige data-analyse. Door middel van simulaties laten we zien dat de blootstelling aan een geneesmiddel zich goed laat voorspellen, zelfs wanneer zeer weinig bloedmonsters beschikbaar zijn, wat traditioneel een punt van aandacht is in de kliniek.

Het belang van de correlatie tussen farmacokinetische parameters en covariaten bij kinderen en de invloed hiervan op het voorspellend vermogen van een model is verder geëvalueerd in **hoofdstuk 5**. Hier simuleren we de blootstelling van geneesmiddelen wanneer deze aan kinderen in een andere leeftijdscategorie worden voorgeschreven dan waarmee het model ontwikkeld is. Dit bleek niet succesvol, wat impliceert dat extrapolaties buiten het bereik van de covariaten dat beschikbaar was bij de ontwikkeling van het model, af te raden zijn. Voorspellingen van farmacokinetische modellen zijn slechts betrouwbaar in geval van interpolaties binnen een subgroep van de originele populatie. Daarnaast lieten we zien dat de puntschattingen van de covariaat (bijvoorbeeld mediaan), welke gebruikt worden voor de mathematische beschrijving van de relatie tussen covariaat en farmacokinetische parameters, gefixeerd moeten worden, zelfs wanneer interpolaties zijn gedaan in een subgroep van de originele populatie.

Samenvattend laat dit onderdeel het belang zien van demografische factoren in de farmacokinetiek voor twee geneesmiddelen die momenteel toegediend worden als eerstelijns therapie bij HIV-geïnfecteerde kinderen. Hiernaast illustreren onze resultaten enkele essentiële randvoorwaarden voor de selectie van covariaten. We raden aan om grotere studiegroepen te gebruiken dan in de huidige klinische praktijk gebruikelijk is. Bovendien hebben we de invloed van een evenwichtige verdeling van covariaten en de implicaties hiervan op het voorspellend vermogen van een farmacokinetisch model laten zien. Op grond daarvan is er geconcludeerd dat het is af te raden een model te gebruiken buiten het bereik van de covariaten die zijn onderzocht gedurende de ontwikkeling daarvan. Deze ontdekking roept verdere vragen op aangaande het wijdverbreid gebruik van allometrische schaling in pediatrisch onderzoek.

12.3. DE EVALUATIE VAN VEREENVOUDIGDE ANTI-RETROVIRALE DOSERINGSSCHEMA'S IN KINDEREN

De mogelijkheid om een geneesmiddel eenmaal daags toe te dienen is vaak een aantrekkelijke optie. Echter, men moet er zeker van zijn dat de blootstelling aan het geneesmiddel die bereikt wordt met een dosering van eenmaal per dag vergelijkbaar is met historische waarden die bereikt werden met een ander doseringsschema. In het tweede deel van dit proefschrift laten we zien hoe een modelmatige benadering kan worden gebruikt om de toepasbaarheid van een alternatief doseringsschema voor lamivudine te evalueren. Lamivudine wordt momenteel eenmaal daags toegediend bij volwassenen terwijl kinderen tweemaal per dag gedoseerd krijgen. In

hoofdstuk 6 gebruiken we simulaties om te beoordelen of lamivudine eenmaal daags toegediend kan worden in een virtuele groep van kinderen tussen 3 maanden en 12 jaar oud. Hiervoor werd het farmacokinetische model gebruikt dat was ontwikkeld en gevalideerd in hoofdstuk 3. Wij hebben aangetoond dat dit doseerregime tot AUC_{0-24h} en piekconcentratie waardes leidt, welke vergelijkbaar zijn met historische data in kinderen bij een tweemaal daagse dosering. Eenzelfde simulatie werd uitgevoerd voor abacavir, dat ook eenmaal per dag toegediend wordt bij volwassena en tweemaal per dag in kinderen. In **hoofdstuk 7** hebben we het in hoofdstuk 4 ontwikkelde farmacokinetische model gebruikt om te beoordelen of de blootstelling van abacavir bij eenmaal daags doseren vergelijkbaar was met een tweemaal daagse dosering. Wederom werd aangetoond dat eenmaal daagse abacavir doseringen in kinderen leiden tot AUC_{0-24h} en piekconcentratie waardes die vergelijkbaar zijn met historische data van kinderen en volwassenen.

Deze resultaten zijn belangrijk voor de verbetering van antiretrovirale therapie in kinderen, omdat eenmaal daags doseren kan de therapietrouw en acceptatie aanzienlijk verbeteren. Daarnaast laat onze benadering zien hoe hypothetische scenario's in virtuele patiënten onderzocht moeten worden. Dit concept is in het bijzonder bruikbaar in pediatrisch klinisch farmacologisch onderzoek. Een van de belangrijkste voordelen is de mogelijkheid om de respons op behandeling te evalueren in een groot aantal kinderen, zonder hen bloot te stellen aan de daadwerkelijke behandeling. Verdere details aangaande de implementatie van virtuele klinische studies worden besproken in het volgende deel van dit proefschrift.

12.4. TROUW AAN HIV THERAPIE: “MEDICIJNEN WERKEN NIET IN DE PATIENTEN DIE ZE NIET INNEMEN”

Het is algemeen bekend dat de continuïteit van de werking van een geneesmiddel afhangt van de correcte uitvoering van een voorgeschreven regime, wat gericht is op een adequate blootstelling aan de werkzame stof tussen de verschillende doses. Onderbrekingen tijdens de therapie kunnen de werking van het medicijn negatief beïnvloeden. De gevolgen van onderbrekingen van de behandeling zijn afhankelijk van het medicijn, de lengte van de onderbreking en de te behandelende ziekte. De uitspraak, toegeschreven aan voormalig Amerikaanse Chirurg Generaal C. Everett Koop, “Medicijnen werken niet in de patienten die ze niet innemen”, vat in deze context het belang van therapietrouw mooi samen.

Om de implicaties van de factoren die de variabiliteit in de werking van een geneesmiddel bepalen goed te kunnen begrijpen is een aanpak nodig die rekening houdt met zowel het effect van ontwikkelingsgroei als van het effect van andere patiënt-specifieke karakteristieken die de farmacokinetiek kunnen beïnvloeden. Bovendien is het belangrijk om te bepalen hoe deze factoren in verband staan met het voorgestelde doseringsschema. De huidige algemene opvattingen over het gebruik van combinatiепreparaten suggereren dat een verminderde doseringsfrequentie de werking van antiretrovirale therapie zou doen verbeteren. Echter, bewijs

van therapeutische equivalentie kan niet worden afgeleid uit bio-equivalentie studies of het vergelijken van farmacokinetische data; de werkzaamheid van combinatietherapie berust op een synergistische werking op farmacodynamisch niveau. Een nauwkeurige evaluatie van veranderingen in het doseringsschema maakt een beoordeling van het effect van therapietrouw van patiënten op de farmacodynamiek noodzakelijk. Verschillen in de patronen van therapietrouw zijn een belangrijke oorzaak van onvoorziene variabiliteit in de blootstelling aan het geneesmiddel, geassocieerd met het falen van de therapie. We hebben laten zien hoe de rol van onvoldedige therapietrouw kwantitatief geëvalueerd kan worden. Hiermee hebben we een kader gecreëerd waarin de vraag beantwoord kan worden hoeveel doses een patiënt kan missen zonder dat dit de uitkomst van behandeling beïnvloedt.

In het verleden zijn naast modelleren andere statistische methoden gebruikt om de gevolgen van therapietrouw aan geneesmiddelen te onderzoeken. De consequenties van partiële therapietrouw aan de antiretrovirale therapie is één van de aandachtspunten in dit onderzoeksgebied. De groep van Pfister et al. integreerde bijvoorbeeld informatie over therapietrouw als covariaat in een farmacokinetisch-farmacodynamisch (PKPDP) model om het effect hiervan op de blootstelling van het geneesmiddel te bepalen. Hiernaast zijn er longitudinale modellen opgesteld om het effect van therapietrouw op de progressie van de HIV-infectie te schatten. Andere onderzoekers maakten gebruik van Markovmodellen en weer andere van stochastische methoden. Naast andere voordelen maakt deze laatste aanpak het mogelijk de respons op elk tijdstip afhankelijk te maken van die op het vorige tijdstip. Het vernieuwende in ons werk is het beoordelen van het effect van imperfecte therapietrouw in een kader waarin alle elementen van een clinical trial simulation gecombineerd zijn. In vergelijking met eerder werk zijn belangrijke elementen geïntroduceerd die voorheen ontbraken, waaronder het gebruik van combinatietherapie, meer realistische patronen van ontrouw aan de therapie (willekeurige onderbrekingen van doses, imperfecte timing van opvolgende doses) en een correlatie tussen therapietrouw en resistentie tegen het geneesmiddel. Om het effect van deze geïntegreerde aanpak te illustreren zijn er drie scenario's geselecteerd voor evaluatie: een enkel geneesmiddel gebruikt als monotherapie, combinatietherapie bestaand uit drie geneesmiddelen en een combinatiepreparaat van drie geneesmiddelen toegediend aan de hand van een versimpeld dosisregime. Deze analyse bevat hiernaast een relatie tussen therapietrouw een kans op geneesmiddel resistentie. Dit kader voor simulaties van klinische studies, waarin verscheidene mathematische modellen zijn geïncorporeerd, maakt het mogelijk een precieze voorspelling van de uitkomst van behandeling te doen. Hierbij kunnen ook voorspellingen gedaan worden voor scenario's waarbij een empirische, gerandomiseerde benadering op praktische en ethische bezwaren stuit.

In **hoofdstuk 8** is de invloed van ontrouw aan de therapie geëvalueerd van drie verschillende klassen van antiretrovirale geneesmiddelen die op dit moment gebruikt worden als eerste keus behandeling bij kinderen (NNRTI, NRTI en gestimuleerde PI). Deze studie is de eerste poging tot

het voorspellen van de uitkomst van therapie door een gecombineerde aanpak waarbij farmacokinetiek, farmacodynamiek en ziektemodellen gelijktijdig worden toegepast. Hiernaast werd een logistische regressie gebruikt om de relatie tussen het aantal dagen waarop de blootstelling op een subtherapeutisch niveau was en de kans op resistentie te beschrijven, wat de schakel tussen onvolledige compliantie en het virologisch falen was. Hierbij hebben we om praktische redenen, in combinatie met een gemakkelijkere interpretatie, de therapietrouw beschreven als patronen van inname van doses in plaats van het percentage genomen doses. Hierbij is het belangrijk te vermelden dat alle gebruikte modellen zijn geëvalueerd en gevalideerd. Onze resultaten geven een voorlopige indicatie van het niveau van therapietrouw dat noodzakelijk om virologisch falen van diverse geneesmiddelen te voorkomen in geval van monotherapie. Deze scenario's vormen een beginpunt voor de evaluatie van de implicaties van slechte of matige therapietrouw in geval van combinatietherapie. Vervolgens werd in **hoofdstuk 9** een virtuele klinische studie uitgevoerd waarbij een combinatie van drie geneesmiddelen werd voorgeschreven in een populatie van HIV-geïnfecteerde kinderen. Hier lieten wij zien dat, bij een op NNRTI gebaseerd regime, een langere periode van onderbreking in de behandeling een hogere kans op virologisch falen kan geven in vergelijking tot wanneer ditzelfde aantal doses willekeurig gemist zouden worden.

De genoemde resultaten laten zien dat de PKPD-relaties van antiretrovirale geneesmiddelen gebruikt zouden kunnen worden om het effect van verminderde therapietrouw, en daarmee ook van versimpelde doseringsregimes, te voorspellen. PKPD-relaties kunnen gezien worden als een surrogaat of proxy van therapeutische equivalentie in de beoordeling van de vergevingsgezindheid van therapieontrouw, vergelijkbaar met de farmacokinetiek in bioequivalentie studies. Dit concept biedt de mogelijkheid om onderzoek naar de effectiviteit van geneesmiddelen te vervangen door PKPD-studies. Zoals weergegeven in **hoofdstuk 10**, werd het effect van partiële compliantie op een versimpeld doseringsregime geëvalueerd met gebruik van de onderliggende PKPD-relaties van lamivudine en abacavir in combinatie met efavirenz. Onze resultaten laten zien dat non-compliantie bij een eenmaal daags regime vergelijkbaar is met het op dit moment goedgekeurde tweemaal daagse regime wat betreft de implicatie van gemiste doses in de onderzochte scenario's van therapieontrouw. Praktisch betekent dit dat een vermindering in doseringsfrequentie kan zorgen voor een vermindering van de medicatielast bij kinderen zonder een toename van het risico op resistentie.

Samengevat verschaft het kader van klinische simulaties, beschreven in hoofdstukken 8, 9 en 10, de mogelijkheid om klinisch relevante informatie te evalueren over het niveau van de therapietrouw dat nodig is om virologisch falen te voorkomen. Bovendien zou dit, in bredere zin, artsen kunnen helpen met het overwegen van het beste dosisregime gebaseerd op het patroon van therapietrouw van de patiënt. Het is vermeldingswaardig dat ter voorkoming van het mogelijk misleidend effect van uitval van patiënten op de uitkomst van therapie dit in de huidige analyses niet is meegenomen. Onze bevindingen suggereren echter dat een slechte therapietrouw

gelijke, of zelfs grotere effecten kan hebben op de resultaten van de behandeling dan uitval van patiënten tijdens een klinische studie.

12.5. CONCLUSIES, AANBEVELINGEN EN PERSPECTIEVEN

In **hoofdstuk 11** zijn de resultaten van de opeenvolgende hoofdstukken van deze thesis samengevat. Onze bevindingen benadrukken de waarde van een modelmatige benadering in pediatrisch onderzoek naar HIV bij vraagstukken die, ondanks de wetenschappelijke en klinische vooruitgang in dit onderzoeksveld, onbeantwoord bleven. Het eerste punt betreft de aanbevolen dosering van antiretrovirale middelen voor kinderen welke, wanneer gebaseerd op overbruggingsconcepten, een vergelijkbare blootstelling geeft op verschillende leeftijden, in tegenstelling tot een dosis gebaseerd op lichaamsgewicht. Hiervoor dienen de groei en ontwikkeling, welke de geneesmiddelblootstelling in de doelgroep beïnvloeden, te worden gekarakteriseerd en gekwantificeerd, om zodoende een blootstelling te bereiken in kinderen die vergelijkbaar is met volwassenen. Hiernaast laat ons werk potentiële beperkingen zien van het gebruik van modellen en simulaties voor het voorspellen van farmacokinetiek in kinderen. We willen om die reden de volgende aanbevelingen voor de implementatie van een modelmatige benadering in pediatrisch onderzoek benadrukken:

- 1) Populatie farmacokinetische studies moeten geschikte inclusie- of stratificatiecriteria overwegen om verzekerd te zijn van een evenwichtige verdeling van covariaten;
- 2) Het gebruik van een meta-analyse, waar verschillende subgroepen van de populatie zijn inbegrepen, verdient een sterke aanbeveling om misspecificatie van het model door misleidende effecten als gevolg van een onevenwichtige verdeling van covariaten te voorkomen;
- 3) Het voorspellend vermogen van een model is beperkt tot interpolaties. De nauwkeurigheid van extrapolaties buiten de populatie die gebruikt is in het bouwen van het model kan niet gewaarborgd worden, zelfs wanneer diagnostische en validatie procedures een bevredigend resultaat geven.
- 4) Extrapolatie tussen populaties buiten de onderzochte condities of populatie vereist het gebruik van simulatiemodellen, welke niet hetzelfde zijn als de modellen die gebruikt worden voor het schatten, zoals in het geval van modellen die ontwikkeld zijn met de maximum waarschijnlijkheid methode.
- 5) Simulatiemodellen zijn essentieel voor het genereren van hypotheses en gevolgtrekking, welke inzicht kunnen geven ook in relevante condities en scenario's die in de praktijk niet waargenomen of gecontroleerd zouden kunnen worden. Zodoende kunnen deze geparametriseerd worden in termen van geneesmiddel-, patiënt- en systeem-specifieke eigenschappen, ongeacht de precisie of identificeerbaarheid, welke gedreven worden door de data.

Gebaseerd op de eerdergenoemde aanbevelingen kan het gebruik van een modelmatige benadering uitgebreid worden naar vraagstellingen die betrekking hebben op het doseringsadvies. Het kan systematisch toegepast worden voor de evaluatie van versimpelde behandelingsopties, hetgeen erg aantrekkelijk is in de pediatrische praktijk. Ongetwijfeld zullen modellen, gecombineerd met simulaties van klinische studies, een hulpmiddel worden voor het opzetten van experimenten.

De aanname dat het genereren van bewijs een sine qua non conditie is voor goedkeuring door de registratie autoriteiten en voor de optimalisatie van het therapeutisch gebruik van geneesmiddelen, zou herzien moeten worden. De belemmeringen voor het opzetten van een protocol en problemen rond de haalbaarheid bij zowel pediatrisch onderzoek als ernstige infectieziekten roept vragen op over de relevantie van het bewijs gegenereerd onder strikte gecontroleerde condities, gezien deze niet het gebruik van geneesmiddelen in de praktijk reproduceren of reflecteren. Het is noemenswaardig dat het leunen op empirische bewijsvoering wordt aangewakkerd door registratie autoriteiten, die richtlijnen blijven uitbrengen waarin deze nieuw gegenereerde bewijsvoering wordt vereist voor de goedkeuring van nieuwe geneesmiddelen.

Dit terwijl de synthese van bewijs (evidence synthesis) op basis van nieuwe en bestaande data in combinatie met een modelmatige benadering, ondanks zijn brede toepassing in de evaluatie van effectiviteit en de analyse van de kosten en baten, niet volledig geaccepteerd wordt in zowel de kliniek als door regulatoire instanties.

Een verschuiving in het paradigma is nodig waarbij synthese van bewijs de voorkeur heeft, waardoor het generen van bewijs een bevestigende stap is in het continuüm tussen aannames en empirisch bewijs. Een van de lessen geleerd van het werk dat in dit proefschrift is gepresenteerd is dat het genereren van bewijs zonder integratie van data, inclusief de systematische incorporatie van voorafgaande kennis, kan leiden tot suboptimale experimentele protocollen en mogelijk verkeerde beslissingscriteria. Dit aspect wordt vaak genegeerd in de rationale voor de reguliere pediatrische klinische studies. Een ander belangrijk punt dat voortkomt uit onze voorbeelden is dat de synthese van bewijs veel krachtiger kan zijn dan het generen van bewijs, doordat het inzicht geeft in condities die niet experimenteel geëvalueerd kunnen worden.

We sluiten deze thesis af door het eerste vraagstuk in de titel te herhalen; maakt een halve dag een verschil op het resultaat van de behandeling? Gebaseerd op de synthese van bewijs door modelleren en simuleren kan worden geconcludeerd dat dit niet het geval is. Sterker nog, het is het onze verwachting dat dit werk zal bijdragen aan het consolideren van het gebruik van PKPD-relaties als de basis voor conclusies betreffende therapeutische equivalentie, waardoor het gebruik van onnodige effectiviteitsstudies in kinderen overbodig wordt.

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor Prof. Oscar Della Pasqua for the opportunities that he gave me in the past six years, his brilliant ideas, his enthusiasm, and above all, for trusting me and believing in my capabilities.

Prof. Danhof, thank you for constantly encouraging and supporting me. Our meetings were always important occasions to receive wise suggestions and useful feedbacks.

I am thankful to the staff, post-doc and PhD colleagues that I got to know during the years spent at the Division of Pharmacology of the University of Leiden, with whom I could exchange ideas and experiences and ask for support and collaboration: Francesco, Li (for always listening to me and giving advices), Roos, Donato, Sven (for trying to teach me Dutch, sorry for being such a bad student!), Vincent, Maurice, Massimo, Elke, Anne, Ibrahim, Rick, Esther, Amit, Flora, Jasper, Joost, Chantal, Massoud, Stephan, Stina. A special acknowledgment to Vincenzo for his help with R scripts and NONMEM codes and for his endless patience and kindness.

The majority of the work presented in chapter 8 of this thesis was performed by a very talented and dedicated student that I had the luck to supervise: Sean Oosterholt. The clinical trial simulations in chapters 9 and 10 were possible thanks to the R package developed by Donato Teutonico, whose help was crucial in the last part of this thesis.

I am very grateful to Marta for doing an excellent job with the layout and cover of this book and to Willem, Sean and Rick for providing the Dutch translation of my thesis.

No agosto 2012 eu tive a oportunidade de passar dois meses do meu doutorado na Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo. Queria agradecer muito a Professora Vera Lanchote pela oportunidade, a ospitalidade e o carinho. Muito obrigada aos amigos do laboratorio: Francine (a sua ajuda foi fundamental quando cheguei no Brasil), Natalicia, Natalia, Glauco, Otavio, Rodrigo, Carol Silva, Leonardo, Ana Leonor, Gabi, Daniela e Carol Vieira. Agradeço também as irmãs e amigos do Vita et Pax, a minha família brasileira: Giovanna, Carol, Ana, Rofela, Victor, Rohit, Marlos e Vinicius.

Anneke and Tarj, thank you for being my paranympths and for being great friends who I can always trust and count on. Carmelo, grazie per le chiacchierate, i consigli, le nostre cene; senza dubbio rimarranno tra i ricordi piu' belli dei miei anni a Leiden.

Grazie a Simone, amico di sempre, che e' stato presente al mio diploma, alla mia laurea e ora anche al mio dottorato. Sono sicura che sara' presente in tutti i momenti importanti della mia vita.

Juny, grazie per il continuo supporto, la pazienza, per aver ascoltato all'infinito le mie presentazioni e per aver finto di interessarti a NONMEM e al PKPD modelling. La tua fiducia in me e' stata fondamentale e mi ha permesso di superare le difficolta'. Grazie perche', anche nei momenti difficili, riesci sempre a farmi sorridere.

Infine, un ringraziamento speciale va ai miei genitori, sempre presenti e pronti a sostenermi. Grazie per avermi insegnato i valori importanti, aver assecondato le mie decisioni e aver sempre creduto in me.

Chiara, 23rd August 2013, Pisa

CURRICULUM VITAE

Chiara Piana was born on the 25th of December 1982 in Acqui Terme, Italy. She attended the Liceo Scientifico (High School) "G. Galilei" in Nizza Monferrato, where she obtained her scientific diploma in 2001. Subsequently she started her training in Pharmaceutical Chemistry and Technology at the University of Pavia, where she obtained her MSc in October 2006. In April 2007 she started working at the division of Pharmacology of the Leiden Academic Center for Drug Research in Leiden under the supervision of Prof. Oscar Della Pasqua for the Task Force in Europe for Drug Development for the Young (TEDDY), a Network of Excellence funded under the Sixth EU Framework Programme for Research and Technological Development. Her work was mainly related to the evaluation of compliance of paediatric clinical trials with regulatory guidelines and the assessment of pharmacogenetics in paediatric drug development. In May 2009 she started her PhD research programme at the division of Pharmacology of the Leiden Academic Center for Drug Research with Prof. Oscar Della Pasqua as co-promoter and Prof. Meindert Danhof as promoter, which led to this thesis. In August 2012 Chiara spent two months at the Faculty of Pharmaceutical Sciences of the University of Sao Paulo, Brazil, to directly contribute to several clinical trials.

Since September 2013 Chiara works as Pharmacometrics Specialist for the Global Compound Development Department of Grunenthal, Aachen, Germany.

LIST OF PUBLICATIONS

Piana, C., Zhao, W., Adkison, K., Burger, D., Jacqz-Aigrain, E., Danhof, M. & Della Pasqua, O. Covariate effects and population pharmacokinetics of lamivudine in HIV-infected children. In press. *British journal of clinical pharmacology*

Piana, C., Zhao, W., Adkison, K., Burger, D., Jacqz-Aigrain, E., Danhof, M. & Della Pasqua, O. A model-based approach for the evaluation of once daily dosing of lamivudine in HIV-infected children. In press. *British journal of clinical pharmacology*

Piana, C., Kliphuis E. & Della Pasqua, O. Lack of compliance of European Public Assessment Reports to guidelines for paediatric drug development before the introduction of paediatric investigation plans. *Clinical Trials* 10, 269-79 (2013).

Piana, C., Zhao, W., Danhof, M., Burger, D., Della Pasqua, O. & Jacqz-Aigrain, E. Population pharmacokinetics of abacavir in infants, toddlers and children. *British journal of clinical pharmacology* 75, 1525-35 (2013).

Piana, C., Surh, L., Furst-Recktenwald, S., Iolascon, A., Jacqz-Aigrain, E., Jonker, I., Russo, R., van Schaik, R., Wessels, J. & Della Pasqua O. Integration of pharmacogenetics and pharmacogenomics in drug development: implications for regulatory and medical decision making in pediatric diseases. *Journal of clinical pharmacology* 52, 704-16 (2012).

Piana, C., De Cock, R., Krekels, E., Danhof, M., Allegaert, K. & Knibbe C. The role of population PK-PD modelling in paediatric clinical research. *European journal of clinical pharmacology* 67, Suppl 1:5-16 (2011)

